OXIDATIVE AMMONOLYSIS OF TECHNICAL LIGNINS AND LIGNITES

by

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Thesis presented in partial fulfilment of the requirements for the degree

of

Master of Forestry (Wood Science) (M.Sc.)

at

the University of Stellenbosch

Date: **March 2003**

Study leader: **Prof. G.R.F. Gerischer**

DECLARATION

I, the undersigned hereby declare that the work contained in this thesis is my own original work and had not previously in its entirety or in part been submitted at any university for a degree.

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ABSTRACT

Today there is renewed interest in using soil improvers with fertilising capability as the use of inorganic fertilisers results in ground water pollution through leaching and formation of nitrates in plant materials. Lignin is an important precursor of humic material in soils and with modification, can be used as a raw material for producing slow release nitrogenous fertilisers. This project focussed on the use of industrial residues such as technical lignins which are produced by the South African pulping industry as well as low grade coals such as lignite occurring in South Africa, for the production of high-grade humic substances like Novihum TM, using the special technology developed by the Institute of Plant and Wood Chemistry of the Technical University of Dresden in Germany. Technical lignins derived from kraft lignin, sugar cane baggase, autohydrolysis lignin with a trade name Sucrolin, a calcium lignosulphonate and South African lignites mined in the Kraaifontein and Brackenfell areas as well as German brown coal were subjected to oxidative ammonolysis with the aim to produce slow release nitrogenous fertilizers.

Curie Point Pyrolysis GC/MS was used to determine the structural composition of the raw materials and products. Oxidative ammonolysis reactions were carried out on a laboratory and a pilot plant scale. Highest nitrogen contents were obtained with Sucrolin lignin followed by the lignite from Germany. The amount of nitrogen that could be fixed with oxidative ammonolysis varied between 1.44 – 10% for the various raw materials. The C/N ratios obtained were in the range between 5 – 34. Marginal differences were observed between the materials modified on lab and pilot plant scales. There were improvements in the total incorporated nitrogen when the raw materials were pre-activated with oxidising agents such as hydrogen perxide and a fungal species, *Phanaerochateae chrysosporium* prior to oxidative ammonolysis. Variable amounts of differently bonded nitrogen forms were obtained for the materials tested due to their structural differences.

OPSOMMING

Daar is deesdae hernude belangstelling in die gebruik van grondverbeteringsmiddels wat ook 'n bemestingsfunksie het, aangesien die gebruik van anorganiese kunsmis besoedeling van grondwater deur uitlogingen insypeling veroorsaak, wat weer tot die vorming van nitrate in plantmateriaal lei. Lignien is 'n belangrike voorloper van humiese materiale in grondstowwe en kan dmv modifisering gebruik word as 'n grondstof vir die produksie van stadigstikstof vrystellende nitrogene kunsmisstowwe. Die huidige projek het gefokus op die gebruik van industriële residue en tegniese ligniene wat deur die Suid-Afrikaanse pulpnywerheid geproduseer word, sowel as lae-graad steenkool soos ligniet wat in Suid-Afrika aangetref word, vir die produksie van hoë-graad humiese stowwe soos Novihum TM. Hierdie produk is mbv spesiale tegnologie deur die Instituut van Plant- en Houtchemie van die Tegniese Universiteit van Dresden in Duitsland ontwikkel.

In hierdie ondersoek is verskeie Suid-Afrikaanse tegniese ligniene soos Kraft lignien, suikerriet bagasse, 'n outohidrolise lignien met die naam van Sucrolin en 'n kalsiumlignosulfonaat sowel as SA ligniete, afkomstig van Brackenfell en Kraaifontein, gebied en Duitse bruinkool, aan oksidatiewe ammonolise onderwerp om sodoende verskillende , stadigvrstellende, stikstofryke kunsmisstowwe te vervaardig.

Curie Punt Pirolise GC/MS is gebruik om die chemiese struktuur van die grondstowwe en produkte vas te stel. Oksidatiewe ammonolise reaksies is op 'n laboratorium en loodsaanlegskaal. uitgevoer. Die hoogste stikstofinhoud is met Sucrolin lignien verkry, gevolg deur die ligniet van Duitsland. Die hoeveelheid stikstof wat dmv oksidatiewe ammonlise bereik kon word, het tussen 1.44 en 10% gewissel. Die C/N verhoudings wat verkry was, het varieer tussen 5 en 34. Marginale verskille is tussen laboratorium - en loodsaanlegsskaal gemodifiseerde grondstowwe waargeneem. Daar was verhogings in stikstofinhoud wanneer die grondstowwe vooraf met oksiderende middels soos waterstofperoksied of swamkultur soos *Phanaerochatae chrysosporium*, aktiveer is. Stikstof is op verskillende maniere en in verskillende hoeveelhede gebind.

ACKNOWLEDGEMENTS

I express my sincere thanks and appreciation to:

1. My study leaders, Prof. G.R.F. Gerischer and Dr. T Rypsta (Department of Wood Science, Stellenbosch University), Dr. Falk Liebner, Prof. Klaus Fischer (both from the Institute of Plant and Wood Chemistry, Technical University of Dresden, Germany) for encouragement and constructive comments during the study.

2. The Jülich Research Centre and the National Research Foundation (Science Liaison Office) for their generous financial support.

3. Mrs. Alta Lopes Da Silva (Department of Wood Science, Stellenbosch University), Frau Wichmann (Institute of Plant and Wood Chemistry, Technical University of Dresden, Germany) for their extensive administrative assistance and support.

4. Mr. Timothy Lesch (Department of Chemistry, University of the Western Cape) for assistance with sample analysis.

5. My family, friends and fellow students for the wonderful support they gave me.

6. Finally, I would like to thank my friends Peter Munyongani, Martin Mkhandawire and family, Nwabisa Khubukeli for the phone calls, Dr. Falk Liebner and family, the coffee crew and the entire staff of the Institute of Plant and Wood Chemistry for the warmth and the love they gave me during my stay in Germany.

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CHAPTER 1

BACKGROUND AND PROJECT AIMS

1.1 BACKGROUND

In many places around the world, including South Africa, large areas of land have been damaged by mining, construction, industrial and military activities. These activities have not only damaged the quality and the structure of the soil, but have also been accompanied by serious pollution problems. In addition to these activities, the excessive exploitation of natural resources such as timber has led to serious losses of land through soil erosion in many parts of the world. In order to reduce soil erosion, and to initialize soil recovery, numerous activities in soil rehabilitation and restoration have been undertaken in many parts of the world. These activities are supported by international organisations such as the UN, which has released a Convention to Combat Desertification.

The success of these activities can only be achieved if they can improve soil conditions in a sustainable way for the establishment of both micro- and macroflora. In order for this to take place there should be a sufficient supply of nutrients as well as microbiologically degradable organic matter in the soil. The quality of the soil organic matter is of great importance for soil development and plant growth. However, the natural formation of the stable humus fractions in impoverished soil is a slow process, and therefore needs to be supplemented with high-grade humic substances.

One possible way in which this could be achieved, would be the use of geogenic resources (e.g. peat) or humus precursors like composts or sterilized sewage sludge, which can accelerate soil improvement. However these are problematic because their compositions frequently vary and the pollutant content and composition often exceeds the specifications laid down by soil protection acts or laws, which regulate the use of such materials. Mineral fertilisers with high nitrogen contents have often been used as well, but they often have led to serious ground water pollution and the presence of nitrates in vegetables. This situation has been even more serious in places where the soil is permeable, and thereby quickly allowing agricultural chemicals to permeate into the soil to the ground water level. In light of these foregoing problems, it is necessary to find technical solutions to accelerate the natural humification process using modified appropriate organic starting materials. Organic based fertilisers with the required composition and properties could be used for this process. Amongst the most promising organic starting materials are technical lignins (and perhaps lignite where it is available) obtainable in huge supply from the pulping industry. Although native lignin is the second most abundant, renewable polymeric component of the biomass

after cellulose, only 5% has been utilised in applications other than energy production in pulp mills. Based on pulp and paper production statistics, about 50×10^6 tons of lignin are produced from woody plants at pulp mills worldwide each year **[1].** Among the 5% used in application other than energy production, are lignosulphonates. Lignosulphonates find applications mainly as binders for animal feed pellets, in bricks, ceramics, and road stabilisers, as dispersants for oil well drilling products, dyestuffs, pesticides, carbon black, water treatment additives etc. The use of soil improving products derived from technical lignites could provide a new approach to maintain an ecological balance by returning an industrial by-product (technical lignin) or redistributing a natural product (brown coal) to the natural bio cycle **[2]**.

1.1 OBJECTIVES

The institute of Plant and Wood Chemistry (IPC) of the Technical University of Dresden (TUD) in Germany has over the years developed a method for production of novel type humic substances using industrial residues such as technical lignins as well as low-grade coal substances such as lignite **[3].** Patent rights have been obtained for a novel technology to produce high-grade artificial humus products by oxidative ammonolysis of technical lignins and coal substances **[4].** The trademark of the basic product is Novihum TM. Current research focuses on the development of the method for the production of Novihum TM on a lab, pilot-plant and eventually on an industrial-scale. It also focuses on testing the material on a small scale under simulated weather conditions in a phytochamber using different types of soils. Large scale testing involves setting up field trials in areas with disturbed soils (mainly through mining and erosion) in Germany, China, United Arab Emirates and Greece using lignite as a starting material **[5, 6, 7, 8, 9, 10].** The Novihum TM technology uses a reaction called oxidative ammonolysis (OA). OA uses ammonia as a nitrogen source and oxygen as an oxidising agent and has the capability of producing *N*-modified lignins with a Nitrogen content of up to 11% under atmospheric pressure conditions and up to 20% under increased pressure. This depends on the laboratory conditions employed and the type and origin of the lignin. *N*-lignins (as the products of oxidative ammonolysis are called) release their nitrogen in a differentiated manner. The ease of release of the nitrogen can be classified as follows: ammonium nitrogen (NH^+_4-N) > amide nitrogen (NH_2-N) > and then the strongly bonded nitrogen (Sob-N). This slow leachability or slow release capability of these products has the potential to significantly reduce ground water pollution caused by easily soluble inorganic fertilisers at present.

The main aim of this project was to investigate the possibility of using industrial residues such as technical lignins, which are produced by the South African pulping industry, as well as low grade coals such as lignite occurring in South Africa, for the production of high-grade humic substances using the special Novihum TM technology developed by the Institute of Plant and Wood Chemistry.

1.3 WORK PROGRAMME

This aim of this project was to:

1. Determine the chemical composition and (structure) of the:

    1.1 South African technical lignins and lignites that were used as starting materials.

    1.2 To compare the South African starting materials with the German starting materials such as the technical lignins and lignites that were used in the production of Novihum TM.

2. Synthesize soil improving agents by oxidative ammonolysis using:

    2.1 South African and German starting materials on a laboratory scale using oxygen under pressure.

    2.2 South African and German starting materials on a pilot plant scale using the standard parameters used for the production of Novihum TM.

    2.3 Pre-activation of the raw materials with oxidising agents prior to oxidative ammonolysis with:

        2.3.1 fungal species *Chrysosporium phanaerochateae*

        2.3.2 hydrogen peroxide

3. Study the products by:

    3.1 Structural characterisation using Curie-Point Pyrolysis GC/MS.

    3.2 Determination of the carbon and nitrogen contents and ratios (C/N ratio).

    3.3 Analysis of the different binding forms of nitrogen.

CHAPTER 2

HUMUS: AN OVERVIEW

2.1 BACKGROUND

The term "humus" dates back to the time of the Romans when it was frequently used to designate soil as a whole. It was later applied to organic matter of soils and composts or to different fractions of this organic matter, as well as to complexes formed by the action of chemical agents upon a variety of organic substances **[11]**. For a long time it was believed that humus results from a prolonged rotting of animal and plant bodies, but since then, several thousands of scientific papers have been written on the humic material, yet much remains to be learned about its origin, synthesis, chemical structure and functions in terrestrial and aquatic environments. Humic substances provide nutrients for organic life on earth (most importantly carbon, nitrogen and phosphorous). They arise from the chemical and biological degradation of plant and animal residues by the synthetic activities of microorganisms. The products formed through these activities tend to be more stable than the corresponding starting materials. Humic substances are predominantly dark coloured to black in colour and acidic, predominantly aromatic, hydrophilic, chemically complex, polyelectrolyte-like materials that range in molecular mass from a few hundreds to several thousands. They are usually partitioned into the following three main fractions: -

(a) humic acid (HA), which is soluble in dilute alkali but is precipitated on acidification of the alkaline extract.

(b) fulvic acid (FA) which is that humic fraction which remains in solution when the alkaline extract is acidified i.e. it is soluble in both dilute alkali and acid;

(c) humin, which is that fraction that cannot be extracted from soil or sediment by dilute base or acid.

These three fractions are structurally similar but differ in molecular mass and functional group content, with FA having a lower molecular weight, containing more oxygen but less carbon and nitrogen, and having a higher content of oxygen containing functional groups (CO_2H, OH, C=O) per unit weight than the other two fractions. Various theories have been developed in an effort to explain the humification process in nature **[12]**. Amongst these theories, the following are the most interesting:

(a) the lignin protein theory; which states that lignin is incompletely utilized by soil microorganisms and can undergo a preliminary series of modifications including loss of methoxyl groups (OCH_3), generation of *o*-hydroxyphenols and termination of side chains to from carboxyl groups (COOH). The *o*-hydroxphenols would further oxidize

to quinones, which are capable of undergoing condensation reactions with ammonia (NH_3), which is produced by the degradation of proteins by microorganisms as well as other N-containing substances in the soil. These would then first form humin, then HA and finally FA.

(b) The polyphenol theory; this is the most widely accepted theory. It states that humic acids originate from polyphenols of lignin. Polyphenols are then converted to quinones by polyphenoloxidase enzymes. Quinones then react with N-containing compounds and polymerise to form humic macromolecules of higher complexity. The order of formation of humic acids would then be FA, then HA and finally humin.

In summary, the components of wood (mainly lignin) and animal matter are the raw materials for humus formation and microbial activity is responsible for their conversion to humus. This overview therefore focuses on:

(i) wood components, the raw materials for humus,
(ii) their biodegradation by microorganisms paying particular attention to lignin,
(iii) microbial pathways and the main organisms responsible for the degradation of wood components.
(iv) humus formation in the natural environment and finally and most importantly
(v) the adaptation of the natural humification pathways for the manufacture of artificial humus with characteristics, which are similar to those of natural humus using a process, called oxidative ammonolysis, and technical lignins and lignites as raw materials.

2.1.1 The structure and composition of wood

Wood is a cellular material, which is composed of elongated cells which are orientated in a longitudinal direction of the stem. These cells are connected to each other by openings, referred to as pits. These cells, varying in shape according to their functions, provide the necessary mechanical strength to the tree and also perform the functions of liquid transport as well as the storage of reserve food supplies. Woody plants belong to seed bearing plants (Spermatophyta), which is subdivided into gymnosperms (Gymnospermae) and angiosperms (Angiospermae). Coniferous woods or softwoods belong to the first mentioned category and hardwoods to the second group **[13]**. Softwoods have a comparatively simple structure and are more uniform in appearance than hardwoods. They are made up of few cell types with

long pointed fibrous cells termed tracheids providing both structural support and conducting pathways in wood. Hardwoods on the other hand, comprise several different cells. Vessels are usually clearly visible with a hand lens in a cross section of the wood. The function of the structural support is carried out by another specialised cell termed the fibre. All of them have the basic two-layered cell wall structure (see figure 1), which is composed of the primary wall (P) and the secondary wall. In between cells, a thin layer composed mainly of lignin called the middle lamella is found **[14]**. The chemical components of the cell wall can be classified into primary components and secondary components. Primary components consist of:

(i) cellulose,

(ii) hemicelluloses and

(iii) lignin.

These components are present in the cell wall and exert important influences on the behaviour of wood through their volume and characteristics. Secondary components can be divided into tannins; volatile oils and resins; gums, latexes and other complex organic compounds and ash **[15]**. Primary components form a greater percentage of the total wood material and play a vital role in humus formation.

2.1.1.1 Cellulose

Cellulose is the main constituent of wood. Approximately 40-45% of dry substance in most wood species is cellulose, located mainly in the secondary wall (see figure 1). It is a homopolysaccharide composed of β-D-anhydro-glucopyranose units, which are linked together by (1→4)-glycosidic bonds (see figure 2). The determination of its average molecular mass by light scattering, in addition to sedimentation-equilibrium (ultra centrifugation) measurements, indicates that the degree of polymerisation (DP) of native cellulose is of the order of 10 000 and lower than that of cotton cellulose (about 15 000). Because of its strong tendency for intra- and intermolecular hydrogen bonding, bundles of cellulose molecules aggregate into microfibrils, which form either highly ordered (crystalline) or less ordered (amorphous) regions. Microfibrils are further aggregated to fibrils. Cellulose has a tight fiber structure, which is created by hydrogen bonds, which results in typical material properties of cellulose, such as the high lengthwise tensile strength and its insolubility in most solvents. Cellulose is soluble in only a few solvents such as cupriethylenediammine (CED) and cadmiumethylenediammine (cadoxen). Cellulose is a

solvent-swollen polymer in contrast to lignin, which in solution occupies a compact structure **[13, 16].**

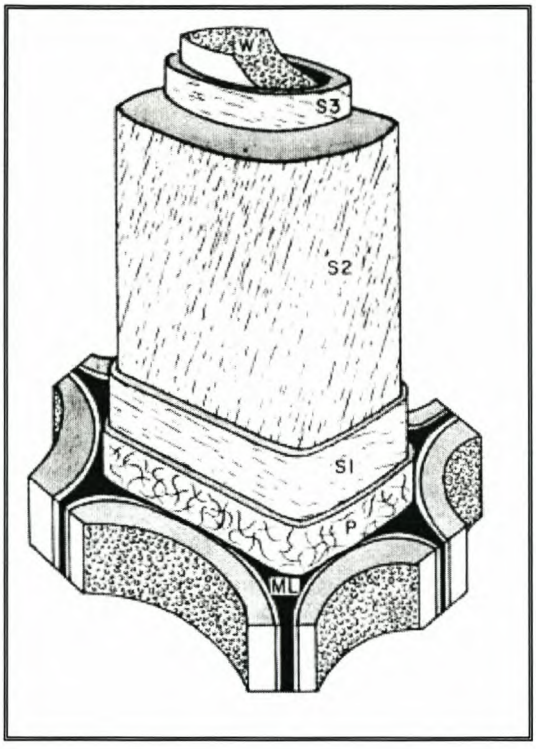


Figure 1: A simplified structure of a woody cell, showing the (ML) middle lamella, the primary wall (P), the outer (S_1), middle (S_2), and inner (S_3) layers of the secondary wall, and the warty layer **[13].**

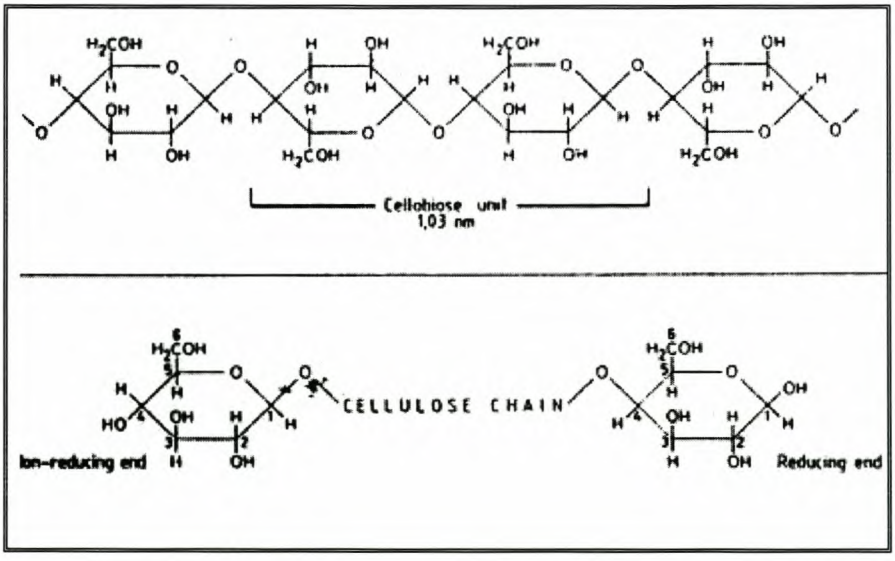


Figure 2: Structure of cellulose, showing a cellobiose unit (the structural repeat unit of cellulose) and the reducing and non-reducing end of a cellulose chain **[15].**

2.1.1.2 Hemicelluloses

Hemicelluloses are short chains of branched hetero-polysaccharides composed of both hexoses and pentoses. D-xylose and L-arabinose are the main constituents of pentosans (xylans), while D-glucose, D-galactose and D-mannose are the main constituents of the hexosans (mannans). The major hemicelluloses component of softwood is mannan-based whilst the hemicelluloses in hardwoods are xylan-based. They comprise 20-25% of hardwood and 7-12% of softwoods. The close association of hemicelluloses with cellulose and lignin in the fiber cell walls contributes both rigidity and flexibility. Hemicelluloses are composed of neutral sugars, all present as their respective anhydrides, e.g., xylan, arabinan, glucan and mannan (substituted with acetyl groups), and of uronic acids. Hemicelluloses in total, constitutes approximately 26% of hardwoods and 22% of softwoods and have a DP of 100 to 200. Apart from galactose-based hemicelluloses, which are characterised by β-1,3-linkages most of the hemicelluloses are built up by β-1,4-linkages between their backbone sugars. The type and amount of hemicelluloses vary widely, depending on plant materials, type of tissues, growth rate, growth conditions, storage and method of extraction. The mannans, galactoglucomannans and glucomannans in softwoods and hardwoods are branched heteropolysaccharides. Xylans appear to be a major interface between lignin and other carbohydrate components in many isolated phenolic-carbohydrate complexes, where they are probably covalent linked to phenolic residues via arabynosil and/or glucoronosyl residues. Xylans tend to absorb onto cellulose and to aggregate with other hemicellulose components, probably as a result of hydrogen bonding. Xylan may play a major role in cell wall cohesion, since its selective removal from delignified wood fiber results in substantial increases in fiber porosity **[14]**.

2.1.1.3 Lignin

Lignin is a cell wall component, which performs various functions that are essential to the life of plants. It plays an important role in the transport of water, nutrients and metabolites by decreasing the permeation of water across cell walls in the conducting xylem tissue. It imparts rigidity to cell walls and, in woody parts, acts as permanent bonding agents between cells generating a composite structure outstandingly resistant towards impact, compression and bending. It also helps tissues to resist attack by microorganisms by impeding penetration of destructive enzymes into cell walls **[17]**. Lignins are therefore partly responsible for giving

plants a chance to conquer the earth's land surface by reinforcing their structural features to such an extent that huge plants such as trees with heights of up to 100m can stand upright **[18].**

Work, which has been done on the chemistry of lignin, indicates that it is a polymer derived from the phenylpropanoid compound (C_6-C_3), coniferyl alcohol and related alcohols (see figure 3) **[15]**. The coniferly alcohol units and related compounds are joined by C-O-C (ether) and C-C linkages.

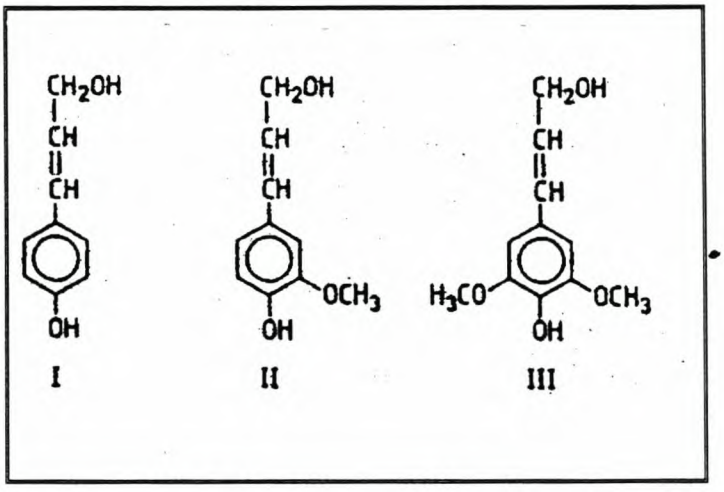


Figure 3: The building units of a lignin polymer: p-coumaryl alcohol(I), coniferyl alcohol (II) alcohol (III)and sinapyl **[15].**

The dominating bond types are depicted in figure 4. The polymeric nature of lignin results from a random combination of the three basic structural units, which is initiated by an enzymatically based oxidation (dehydrogenation) **[19].** This results in the formation of two different types of lignins depending on the species and the position in the cell wall. All angiosperms, including grasses, mainly have guaiacyl-syringyl lignins (co-polymers of synapyl and coniferyl alcohols), while gymnosperms have the guaiacyl type of lignins (polymerisation products of coniferyl alcohols) **[20]**. The designation of the two lignins refers to the different methoxy contents in the aromatic ring structures of lignin (see figure 5).

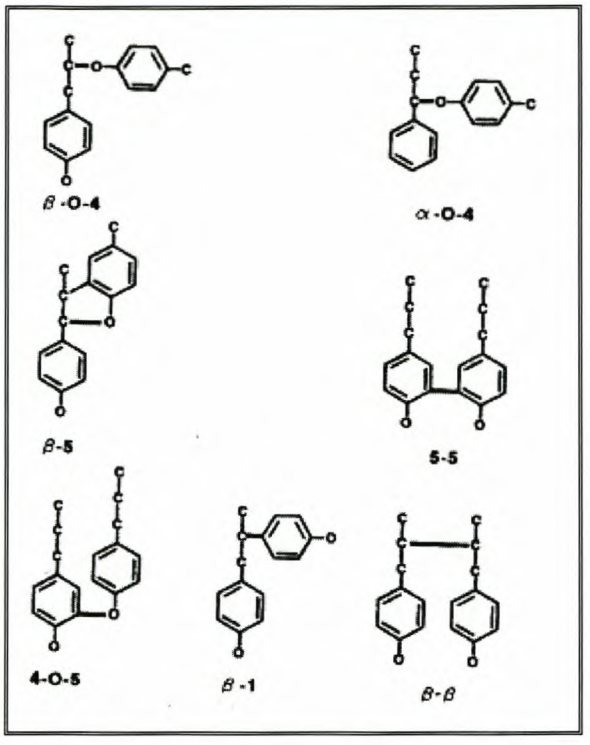


Figure 4: Common linkages between the phenylpropane units **(13).**

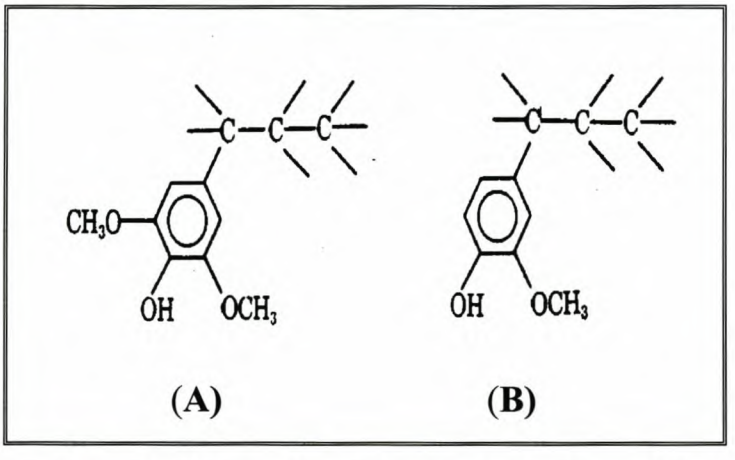


Figure 5: (A) Guaiacyl and (B) syringyl units of lignin **[1]**

Substantial effort has been undertaken in establishing the exact structure of lignin. However this has always been hampered by the fact that lignin is structurally associated with cellulose and other cell wall components. In addition to this, most lignin is mainly obtained as a residue in the isolation of cellulose from wood. The methods employed in this process always seek to dissolve the lignin. As a result of this, it is always isolated in an altered and somewhat degraded form. At the present moment only milled wood lignin (MWL) is believed to be representative of lignin in an unaltered form. Figure 6 presents a hypothetical structural formula of a lignin segments proposed by Adler **[21]**. In wood, lignin is found in its highest

relative abundance in the middle lamella i.e. the lignin content of this layer is high, but because this layer is thin (0.1-1.0μm in thickness), only 20%-25% of the total lignin in wood is found here. Most of the lignin is found in the secondary wall of the cell wall due to the large comparative volume of this layer compared to other layers.

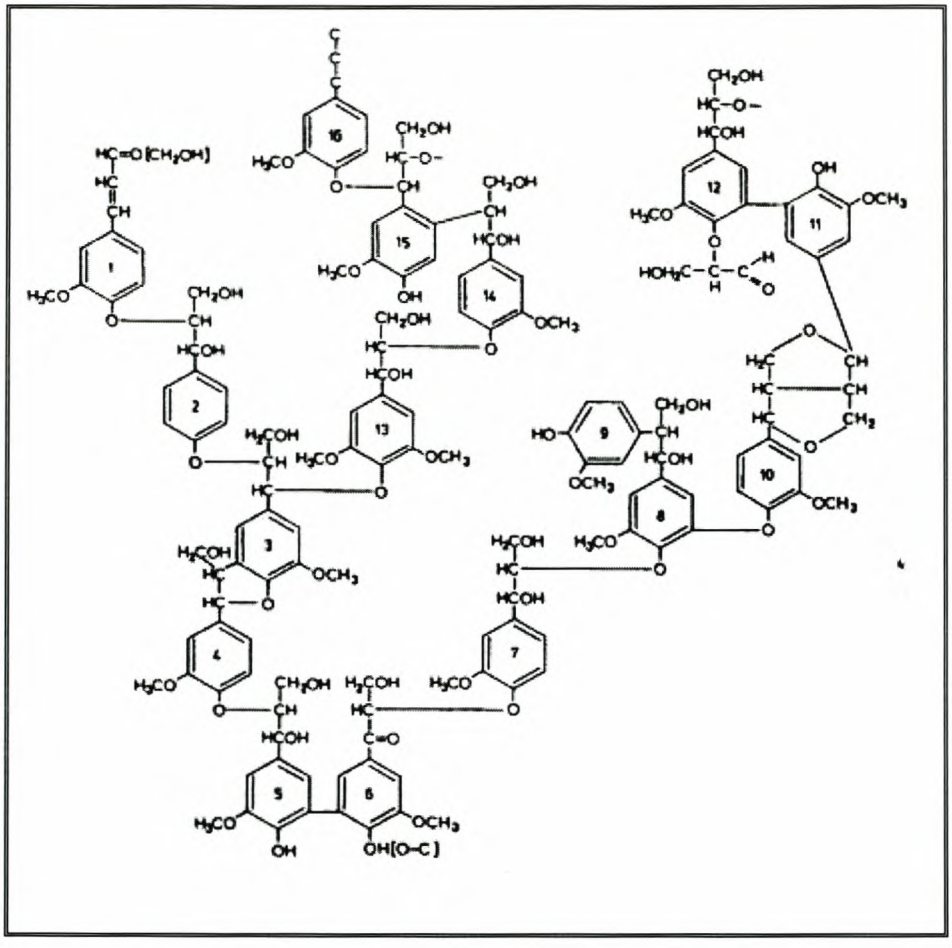


Figure 6: A structural segment of a softwood lignin as proposed by Adler **[21].**

The primary wall is a thin layer, 0.1-0.2μm, and is also composed of lignin embedded between cellulose, hemicelluloses protein and pectic substances (see figure 1 and figure 7) **[15]**.

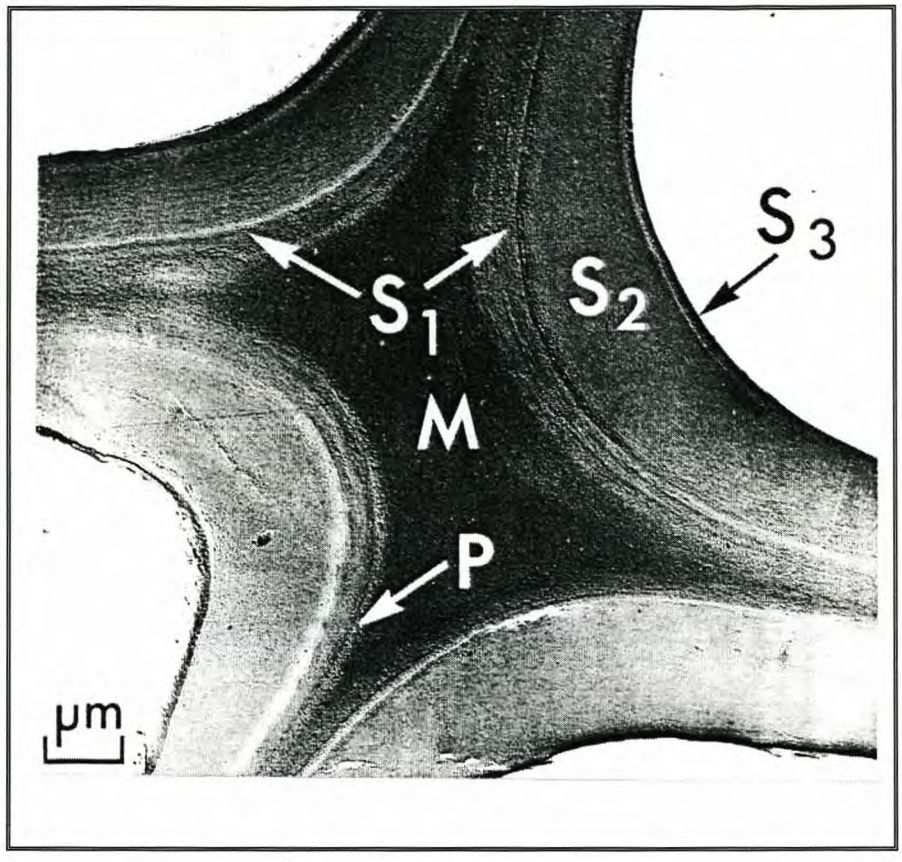


Figure 7: Transverse section though tracheids showing the primary wall (P), the middle lamella (M) and three secondary wall layers (S_1), (S_2) and (S_3) **[13].**

2.1.1.4 Other cell wall components

It has already been mentioned before, that wood plant cell walls, other than cellulose, hemicelluloses and lignin, contain extraneous substances. Many of these substances are extractable with neutral solvents and are referred to as extractives of which there is a considerable variation in the distribution as well as the amount in which they are found in any given tree. Sugars and other sap-soluble constituents, and deposited reserve foods such as starch are found in the cell lumens of parenchyma cells. Phenolic materials are, however, deposited in the heartwood. Fats are found in the parenchyma cells, especially in the ray parenchyma, whereas resins are secreted by epithelial cells and tend to form resin ducts. Coloured and volatile constituent provide aesthetic characteristics. Certain phenolic compounds lend resistance to fungal and insect attack and with resulting durability, and silica imparts resistance to the marine borer. Some extractives are utilized commercially such as vegetable tannins, turpentine and tall oil; rosin fatty acids etc. Some have negative properties such as alkaloids, which may present health hazards. Extractives have been classified into

various groups on the basis of certain structural features, but there is often overlapping because of the polyfunctional nature of these compounds **[22]**.

2.1.2 Biological degradation of wood

Higher plants play an important role as raw materials for the formation of humus. These organisms synthesize vast quantities of insoluble aromatic macromolecules including tannins and most importantly for this discussion, lignins. These macromolecules are biodegradable, but their degradability is slow. This slow decomposition is probably the rate-limiting step in the biospheric carbon-oxygen cycle (requiring several thousands of years for the complete recycling of polyaromatic molecules complexed into soil humic substances). This step is mediated by catabolic activities of microorganisms **[23]**.

When vascular plants die or drop litter, lignified carbon is incorporated into the top layer of the soil. This material has to be broken down and recycled by microorganisms to maintain the earth's carbon cycle. The microbial degradation of litter results in the formation of humus, and lignolysis probably facilitates this process by promoting the release of aromatic humus precursors from the litter. The organisms principally responsible for lignin degradation are aerobic filamentous fungi, and the most rapid degraders of this group are Basidiomycetes. The ability to degrade lignin efficiently is thought to be associated with mycelia growth habit, which allows the fungus to transport scarce nutrients e.g. nitrogen and iron, over a distance into the nutrient poor lignocellulose substrate that constitutes its carbon source. Actinomycetes (i.e. bacteria with a mycelia growth habit) have not evolved the capability to degrade lignin efficiently. It is possible that they have the ability to modify lignin somewhat, but no evidence has accumulated to show that they can degrade lignin as well **[24]**.

2.1.2.1 Fungal decay of wood

In wood, three distinct types of fungal decay can be distinguished i.e. white rot, brown rot and soft rot. White rot and brown rot are distinctive in their visual and structural appearance. White rots typically cause bleaching of wood, which acquires a fibrous spongy consistency. They may further be subdivided either according to their distribution of decay (e.g. white pocket rot, white ring rot) or pattern of lignin removal (simultaneous rot and selective delignification) **[25]**. White rot fungi degrade and metabolise all the major cell wall constituents, proceeding from the lumen surface inward while leaving the residual cell wall

material intact. Wood degraded by white rot fungi is characterised by low alkaline solubility and only minor reduction in the DP of the residual cellulose. White rot fungi vary in the degree of the early attack on lignin and cellulose. Strength losses in white rotted wood are proportional to specific gravity reductions. Brown rots usually result in brown discolouration of the wood accompanied at the late stages by cubical cracking and acquisition of a friable consistency. Brown rots generally degrade all the major structural cell wall components of wood, including cellulose although lignin may in some cases be removed preferentially. Brown rot fungi develop rapidly in the S_2 and S_1 (see figure 1) zones of the cell wall causing dramatic reductions in the DP and increasing alkaline solubility. The changes are also associated with early, drastic reductions in wood strength properties.

Soft rot fungi are slower and less aggressive decayers than white and brown rot fungi, and are probably less important degraders in a quantitative sense. They concentrate their attack on the cell wall carbohydrates. They cause more degradation to lignin than the brown rot fungi. Soft rot degradation is primarily localised in the S_2 layer of the cell wall. As with the white rots, soft rotted wood is characterised by only minor reductions in alkali solubility **[26]**.

2.1.2.2 Fungal biodegradation of lignin

The study of lignin biodegradation is very important to the biotechnological production of useful substances from lignin. Humus and humus formation is no exception to this rule as these studies provide valuable knowledge as far as lignin degradation pathways involved in humus formation are concerned. It further provides information on the microorganisms involved, as well as the degradation products which are formed upon condensation with nitrogen containing substances from humic substances. Substantial effort has been put into the study of lignin biodegradation and in this regard the white-rot (see previous discussions) fungal species, *Chrysosporium phanaerochateae,* has received tremendous attention. To date, the most plausible contribution to the study of lignin biodegradation comes from radioactive labelling studies of lignocellulosics (^{14}C-lignins). These studies showed that in as much as lignin is a formidable substrate for microbial catabolism, it does not serve as an energy source for microorganisms. Studies by Haider **[27]** (see Table 1) showed that ^{14}C-labelled glucose or cellulose loose about 80-90%, and wheat straw about 70% of their carbon during incubation periods of one year. Lignin, however, loose only about 30% of their structural carbons during one year and 40-45% in two years.

Table 1
Biodegradation and incorporation into biomass and 6 N HCl hydrolyzable portion after incubation of soils (Typic Hapludalf and Mollic Haploxeralf) for different months with various ^{14}C-labeled organic compounds

	% ^{14}C-CO_2 evolved				% ^{14}C in biomass[a]		% ^{14}C hydrolyzed[a]	
	Months							
Compounds	1	6	12	24	12	24	12	24
Glucose (UL ^{14}C)[b]	70	85	89	-	19	-	72	-
Wheat straw polysaccharide (UL ^{14}C)[b]	55	78	81	84	10	8	60	58
Wheat straw (UL ^{14}C)[b]	31	63	69	71	7	5	56	48
Lignin[c] ^{14}C-ring	5	26	33	45	0.5	0.4	19	18
Lignin[c] ^{14}C-side chain	7	28	34	42	0.5	0.4	21	19

a) % of residual ^{14}C in soil
b) uniformly ^{14}C-labeled
c) cornstalk material with ^{14}C label in the lignin portion at aromatic rings or at C-β of the side chains.

From these studies conclusions could be reached that microorganisms in soils only use polysaccharides (and also sugars, proteins and derivatives) as carbon and energy sources for the synthesis of their biomass whereas lignin cannot be used for these purposes. Further studies by Haider **[28]** confirmed that even though lignin is not readily available as a carbon and energy source, lignin derivatives and other phenolic substances are readily utilized as carbon sources at concentrations up to 1% or more by soil microorganisms. Studies by Kirk **[29]** showed that lignin metabolism by *Chrysosporium phanaerochateae* occurs in a simple, defined medium, but that control of several culture parameters – particularly O_2, nutrient N, and pH – is essential for maximum rates. Other studies involving ^{14}C-labelling also showed that lignin is biologically eroded, especially in the presence of air, whilst biologically inert in the absence of molecular oxygen **[30]**. This in turn has environmental implications in the sense that, the absence of aerobic lignin degradation leads to the accumulation of lignin and lignin derived materials, which over extended periods of time form the basis for coal and peat. Radioactive ^{14}C labelling studies in lignin biodegradation have alleviated many problems that have plagued lignin biodegradation studies in the past **[31]**. However, there are important limitations to them. Firstly, they do not take into account the degradation of lignin into water-soluble intermediates. Secondly, the radioactive labelled synthetic lignins are still expensive and not readily available. Furthermore, care should be taken to ensure that the synthetic lignin

preparation is polymeric, which is a potential problem with DHPs (Dehydrogenative Polymerizates). Polymeric dyes, such as poly R, poly B, remazol blue, and others have been used as indicators of lignin degradation but the dyes must be verified by studying the degradation of [^{14}C] DHPs, milled wood lignin and other substrates **[32]**.

2.1.2.3 Chemistry of lignin degraded by white-rot fungi

Much of the knowledge about the chemistry of lignin degradation by white-rot fungi comes from studies of fungal degraded polymeric lignins isolated from decayed wood compared with products obtained from sound wood **[33]**. Chang et al **[34]**, in their studies of the lignin degradation pathways by white rot fungi, found that, lignin degradation reactions are mainly oxidative since the oxygen contents of the isolated polymeric lignins are generally higher than the corresponding lignin from sound wood. The oxidative reactions are accompanied by the formation of substantial amounts of carboxylic acids, both aromatic and aliphatic, in the lignin polymer (see figure 8 below).

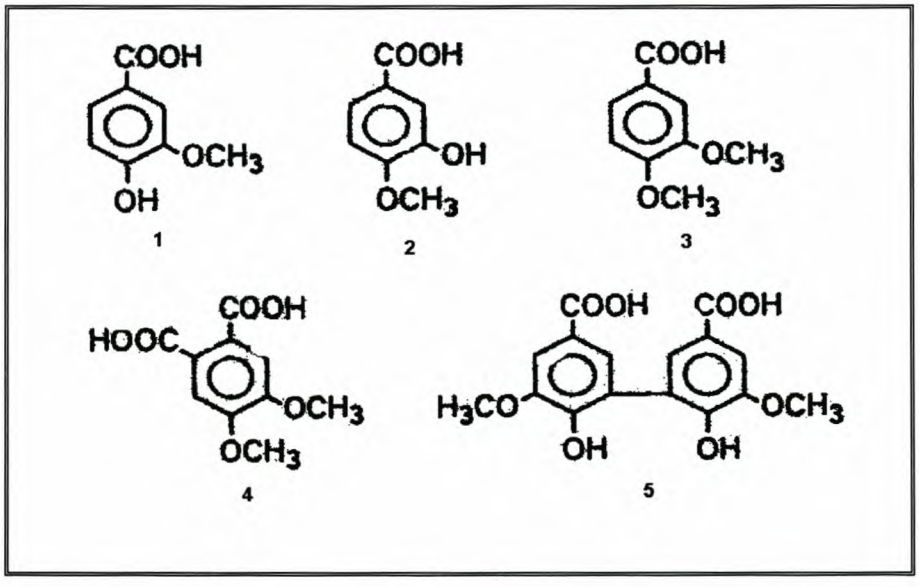


Figure 8: Carboxylic acids resulting from lignin degradation i.e. 1. Vanillic acid; 2. Isovanillic acid; 3. Veratric acid; 4. m-hemipinic acid and 5. Dehydrodivanillic acid **[32].**

These compounds are formed by oxidative splitting of side chains (Cα – Cβ cleavage and Cα oxidation) with the formation of aromatic carboxylic functions, cleavage of β-aryl ethers and

oxidative modifications of side chain structures and probably ring cleavage to carboxylic acid groups. Some of the ring cleavage products are presented in figure 9 and may come from 5-5 (biphenyl) and β-O-4 (β-aryl ether structures).

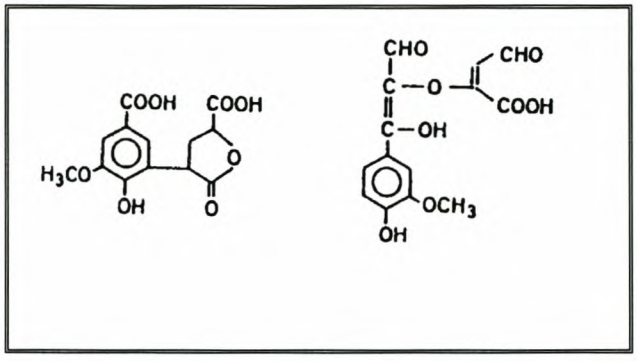


Figure 9: Two ring cleavage products of wood degraded by *P. chrysosporium* **[32].**

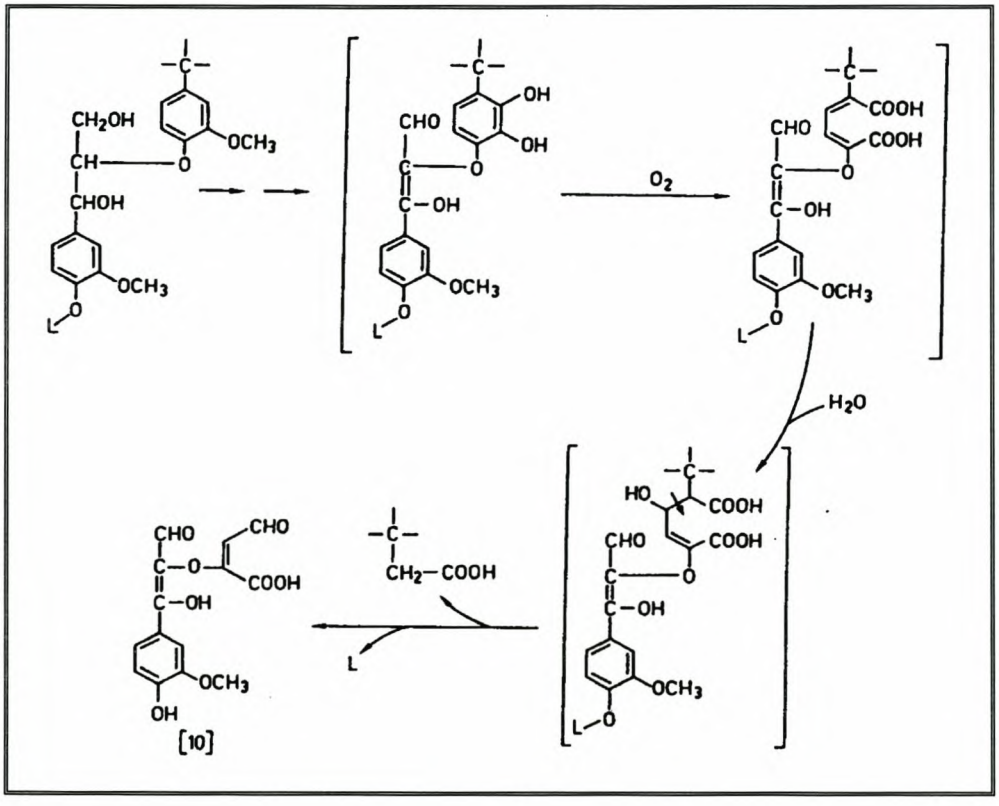


Figure 10: Proposed scheme of lignin degradation to form one of the ring cleavage structures in figure 9 **[32]**.

A lignin polymer, which has been degraded by white rot fungi, contains less carbon, methoxyl and hydrogen than lignin obtained from sound wood **[32]**.

2.1.2.4 Biochemistry of lignin degraded by white-rot fungi

An enzyme participating directly in lignin degradation was discovered in the 1980s. This enzyme, named lignase or lignin peroxidases was discovered in the fungal species *P. chrysosporium*, and two independent research groups published the first results on this new enzyme on the same date namely August 12, 1983. Further studies on this enzyme involving the separation of 21 extracellular proteins of *P. chrysosporium,* revealed that 15 of the proteins oxidized verartryl alcohol, while six were Mn dependent. This led to the designation of two peroxidases enzymes i.e. (i) lignin peroxidases (LiP) and (ii) the manganese dependent peroxidases (MnP) **[33]**. Since the discovery of LiPs, a series of other lignolytic enzymes have been isolated. According to their typical production patterns of extracellular enzymes, white rot fungi can be divided into three groups i.e. (i) LiP – Mn group (ii) MnP – laccase group and (iii) the LiP – laccase group, although overlaps and exceptions certainly occur **[34].**

2.1.2.4.1 Lignin peroxidases

Both LiPs and MnPs contain a ferric heme and operate via a typical peroxide catalytic cycle. That is, LiPs for example are oxidized by H_2O_2 to a two-electron deficient intermediate, which returns to its resting state by performing a two electron one-electron oxidations of donor substrates. However LiPs are more powerful oxidants than typical peroxidases, and consequently oxidise not only the usual peroxidases substrates such as phenols and anilines, but also a variety of non-phenolic lignin substrates and other aromatic ethers that resemble the basic structural unit of lignin. The simplest aromatic substrates for LiPs are methoxylated benzenes and benzyl alcohols, which have been used by enzymologists to study LiP reaction mechanisms. The LiP catalysed oxidation of lignin substrates begins with the abstraction of one electron from the donor substrate aromatic ring, and the resulting species, an aryl radical then undergoes a series of post enzymatic reactions. For example, dimeric model compounds that represent the major aryl glycerol-b-aryl ether lignin structure, undergo $C\alpha - C\beta$ cleavage upon oxidation by LiP. This is in support of the lignolytic role of LiP because $C\alpha - C\beta$ cleavage is the major route for lignolysis by many white rot fungi.

2.1.2.4.2 Manganese peroxidases

MnPs, as mentioned earlier are, similar to LiPs except that Mn (II) is the obligatory electron donor for the reduction of the one electron deficient enzyme to its resting state, and Mn (III) is produced as a result. This reaction requires the presence of bidentate organic acid chelators such as glycolate or oxalate, which stabilise Mn (III) and promote release from the enzyme. The resulting Mn (III) chelates are small, diffusible oxidants and are consequently unable to attack the relactrant non-phenolic structures that predominate in lignin. However, Mn (III) chelates do oxidise the more reactive phenolic structures that make up approximately 10% lignin. These reactions result in a limited degree of lignolysis via $C\alpha$ – aryl cleavage and other degradative reactions.

2.1.2.4.3 Laccases

Laccases are blue copper oxidases that catalyse the one-electron oxidation of phenolics and other electron-rich substrates. Most lignolytic fungi produce laccases, *P. chrysosporium* being a notable exception. Laccases contain multiple copper atoms, which are reduced as the substrates are oxidized. After a laccase molecule has received four electrons, the laccase reduces molecular oxygen to water, returning to its native state. The action on lignin resembles that of Mn (III) chelates, in that units are oxidized to phenoxy radicals, which can lead to degradation of some structures. In the presence of certain artificial auxiliary substrates, the effect of laccase can be enhanced so that it oxidises non-phenolic compounds that otherwise would not be attacked, but it is not yet known whether natural versions of such auxiliary substrates function *in vivo* in lignin biodegradation **[35]**.

2.1.3 Summary

In summary, the above discussion has a lot of significance in the study of humus and the humification process. It serves as a benchmark for the understanding of the chemical constituents of humus and the biological organisms and the pathways they follow in manufacturing this complex material. This is mainly due to the fact that the fats, waxes and resins in the soil, the carbohydrates and the proteins; the lignins and lignin-like materials, all have their counterparts in plant material, although the corresponding groups may not be identical in chemical composition. This variation is associated with the transformations of the complexes of plant origin in the soil through the activities of microorganisms; some of the plant constituents become completely decomposed, others are modified to a greater or less

extent, and still others are more slightly attacked. These processes are accompanied by the synthesis of new complexes (fats, carbohydrates, proteins, and even lignin-like compounds) by microrganisms.

2.2 HUMUS

Humus formation may take place mainly by reaction of mineral acids with carbohydrates to form furfurals, condensation of amino acids and peptides with carbohydrates to form melanoids, aldol condensation of amino acids with methylgyoxal, oxidation of phenol quinones and other aromatic compounds, and opening of lignin rings **[36]**. Figure 11 below is a scheme illustrating the natural humification of lignin. This process takes place both under aerobic and anaerobic conditions usually in soils, composts, peat bogs, and water basins. Microbial processes are responsible for most of humus formation. They contribute to the formation of humus by two processes: one is the extracellular transformation of plants and animal constituents into humic compounds, a process which primarily involves lignin humification. The other is the synthesis of humic precursors within the cells starting with simple aliphatic compounds. This synthesis is linked to the fundamental metabolism of microbes and indicates that humus may be formed as a metabolic by-product of carbohydrate metabolism **[37]**.

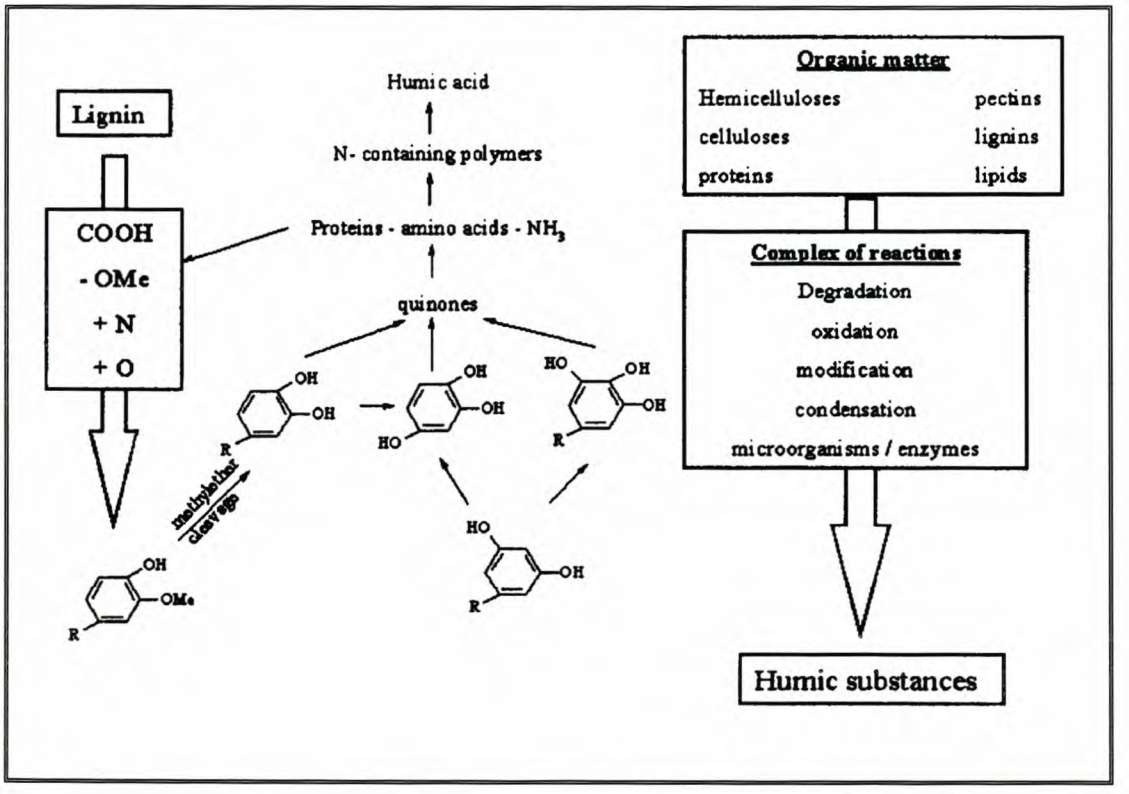


Figure 11: Reaction scheme for the natural humification of lignin **[33]**.

Humic substances, by virtue of their origin from plant materials, chemically consist of certain constituents of the original plant material (e.g. lignin degradation products), which are resistant to further decomposition, of complexes resulting from the decomposition, either by processes of hydrolysis or oxidation and reduction, and various compounds synthesized by microorganisms. The functions of humus in the soil are largely threefold:

(i) physical, thereby modifying the soil colour, texture, structure and moisture holding capacity and aeration;

(ii) chemical, influencing the solubility of certain minerals, forming compounds with certain elements such as iron, which renders them more readily available to plants, and increasing the buffering properties of the soil;

(iii) biological, by serving as a source of energy for the development of microorganisms, as well as making the soil a better medium for the growth of higher plants; it also supplies a slow but a continuous stream of nutrients for plant life.

2.2.1 Humus Types

Various classifications of humus have been proposed and four distinct types have been recognised:-

(i) the brown variety, found in living vegetation, in recently fallen litter, in peat, in decomposing sea weeds upon the shores and fungi.

(ii) the black variety found in an active state of decomposition in the deeper layers of the soil, in decomposing leaves and wood of forests, in animal manures, in peat swamps and in muds.

(iii) humus, in the state of transference, namely in the waters of rivers, lakes, springs, and even in rainwater;

(iv) humus, in the form of fossils, in the form of lignite, brown coal and other carbonaceous deposits.

2.2.2 Characteristics of humus

Some of the characteristics have already been mentioned, but the following are worth mentioning as well:

1. Humus contains a somewhat larger amount of carbon than do plant, animal and microbial bodies; the carbon content of humus is usually about 55 – 56% and frequently reaches 58%.

2. Humus contains considerable nitrogen, usually about 3 – 6%. The nitrogen concentration may be frequently less than this figure; in the case of highmoor peats, for example it may be only 0,5 – 0,8%. It may also be higher, especially in subsoils, frequently reaching 10 – 12%.

3. Humus contains the elements carbon and nitrogen in proportions that are close to 10:1; this is true in many soils and of humus in sea bottoms. This ratio varies considerable with the nature of humus, the stage of its decomposition, the nature and the depth of the soil from which it was obtained, and other climatic and environmental conditions under which it was formed.

4. Humus serves as a source of energy for the development of various groups of microorganisms, and, during decomposition, gives off a continuous stream of carbon dioxide and ammonia.

5. Humus is characterized by a high capacity of base-exchange, of combining with various other inorganic soil constituents, of absorbing water, and of swelling and by other physical and physico-chemical properties which make it a highly valuable constituent of substrates which support plant and animal life.

2.2.3 The chemical nature of humus

The chemical structure and reactions of humic materials have been the subject of numerous investigations for over 200 years, while much remains to be learned about these materials. Over the years, the availability of advanced and sophisticated instruments has assisted in generating important information on the "building blocks" that make up humic materials. However, little is known on how these "building blocks" align and what structural arrangement they produce. Nevertheless, the available methods for characterisation of humic acids provide reliable information on elemental and functional group compositions. Schnitzer

et al **[38]**, in their studies on this subject, classified analytical methods into degradative and non-degradative methods. Amongst the non-degradative methods are the following:

1. Spectroscopy in the UV visible and I.R. regions;
2. Nuclear Magnetic Resonance and Electron Spin Resonance Spectroscopy.
3. X-ray analysis electron microscopy and electron diffraction.
4. Viscometry and molecular mass distribution.
5. Vapour pressure osmometry.
6. Ultracentrifugation.
7. Gel filtration and electron titrations.

Among the non-degradative methods are gel chromatography and other chromatographic systems which will be described in more detail later. With the aid of these methods, significant advances and in the knowledge of the chemical structure and reactions of humic acids are now possible **[39]**. Degradative methods are useful tools in the study of humic materials as far as chemical structures are concerned. With these methods, complex materials are degraded into simple compounds which can be identified and whose chemical structures can be related to those of the starting materials. Recent advances in the development of efficient gas chromatographic mass spectroscopic systems, that make possible the separation and the qualitative and quantitative identification of micro-amounts of organic compounds in complex mixtures, have greatly improved the efficacy of chemical degradative methods as tools for structural analysis. Included in these methods are alkaline and acidic oxidations, reduction, hydrolysis, thermal, radiochemical and biological degradations **[40]**.

2.2.3.1 Characterisation of humic matter and lignocellulose by Pyrolysis GC/MS

This method has proved to be useful in the characterisation of polymers for the structural elucidation of non-volatile organic compounds. The use of this method dates back as early as 1963. Recently, Hemfling and Schulten **[43]**, in their studies of organic matter of forest soils (characterisation of organic constituents from different forest humus layers) have found that the combination of Curie point pyrolysis GC/MS and Pyrolysis Flame Ionisation Mass Spectroscopy, provides fundamental information, the chemical composition of humus. Amongst the substances they were able to identify include pyrolysis products with molecular

masses of up to 450 Daltons that derive from carbohydrates, intact and degraded lignins, proteins, lipids, polyphenols, and aliphatic polymers. Faix et al **[41]** also successfully separated up to 82 degradation products of lignin and characterised them by mass spectroscopy. In subsequent studies, they were able to characterise degradation products derived from polysaccharides as well. Other studies that have been conducted applying this method include studies on lignocellulosics **[42]**. Through these studies, some advantages and disadvantages of this method were established. The pyrolysis GC-MS analyses of agricultural by-product(s) subjected to biological processes of delignification, and recycled papers were used to show the amount of information that pyrolysis GC-MS can provide on lignin classification and monitoring of delignification treatments as well as on finger printing of lignocellulosic materials. Schulten et al **[43]** in their most recent studies using Curie point pyrolysis GC/MS with a nitrogen selective detector were able to characterise the structure of organic N compounds in four mineral soils. This technique was found fast, sensitive, and suitable for highly specific identification of N containing compounds from whole soils with total N contents of 0,08 and 0,46%. In order to optimise the method, they pyrolysed one agricultural soil at final temperatures of 573, 773 and 973 K. Almost no chemical alterations to identifiable pyrolysis products were observed when the final pyrolysis temperature was increased from 573 to 973 K. More than 50 N-containing pyrolysis products were identified, and were divided into classes characterised by specific molecular structures. These included pyroles, imadazoles, pyrazoles, pyridines, pyrimidines, pyrazines, indoles, quinolines, N-derivatives of benzene, alkyl nitriles and aliphatic amines. Humic acids extracted from soils of very different origins have been analysed by Py-GC/MS in the presence of tetramethylammonium hydroxide (a deravatizing agent) **[44]**. The thermal degradation products identified from these studies consisted mainly of aliphatic series such as fatty acids, methyl esters and α, ω-methoxy fatty acids methyl esters. In some samples tripenoid compounds with ursane, oleane and hopane skeletons were detected. Aromatic units derived from lignin moieties were also detected in several samples although in minor amounts, mainly guaicyl and syringyl units. The tetramethylammonium hydroxide procedure has a capability of releasing the more labile, aliphatic moieties attached to the aromatic nuclei of HA's. Other contributions came from Lars Carlsen et al **[45]** in the studies of classification of coal from different origins. They employed a new method called Flash prolysis GC/MS. This method, in combination with statistical methods such as the Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) proved to be advantageous. These are just some of the many possibilities of applications of prolysis GC/MS in the study of HA's using degradative techniques such as GC/MS.

CONVERSION OF TECHNICAL LIGNINS INTO SLOW RELEASE NITROGEN FERTILISERS

2.3.1 Background

Technical lignins have not occupied a proper place as raw materials for the production of carbon based products. Their utilisation has been hampered mainly by the fact that, their structure, and hence their properties vary depending on the method of isolation. However, today there is a renewed interest in using them as chemical feedstocks in ecofriendly and cost effective processes to produce soil improvers with fertilizing capability. This is mainly triggered by the fact that lignin, by virtue of its position in nature as a precursor of humus and coal – predestines its application for enhancing soil fertility and soil decontamination. This premise has been taken as the basis for development of research activities and technologies aimed at designing novel products for agricultural needs as well as the production of other carbon based materials **[46]**. These efforts, of which some are listed in Table 2, have led to the development of a novel process for the production of lignin substances with fertilising capability. In this process, lignin is enriched with nitrogen by modifying it with ammonia in the presence of oxygen and pressure. Low-grade coal deposits (e.g. brown coal) in central Europe (and perhaps in South Africa) could also be used as chemical feedstocks for this process.

Table 1: Ammoxidation of lignin and black liquors from pulping industries [46]

Scale	Feedstock	Reactor type	Temperature (°C)	Time (min)	Pressure (MPa)	Oxidizing medium	N (wt%)	Remarks
Pilot plant, 500 t/year	Ca-LS	Flow-through	110–130		1·0–1·3	O_2, NH_4OH	18–22	
Laboratory	SSL	2-liter batch autoclave	90–120	240–600	0·8	NH_3, O_2	19	
Laboratory	Ammonium-LS	Batch with air flow (400 ml/h)	160	240–360	8·0	9% NH_4OH	n.a.	41% ammonia, 11·1% amides, 47·9% other N-functionalities
Laboratory	Hydrolysis lignin	Batch	20	n.a.	0	NH_4NO_3 or urea/$KMnO_4$	n.a.	n.a.
Laboratory	SSL ammonium bisulfite	Batch with air flow (5 liters/h)	125–130	5–60	8·0	NH_3	n.a.	Direct relationship between N-content, oxygen consumption, and decreasing amount of methoxyl groups
Laboratory	Fermented SSL ammonia bisulfite	Tube	70–80	480–660	0	NH_3/air or oxygen	10	47% ammonia, 12% amide, 41% strongly bound
Laboratory	Kraft black liquor from Eucalyptus	5-liter batch autoclave	20	45	1·0	Nitric acid (10%)	n.a.	pH too low, unusable product
Laboratory	SSL ammonium bisulfite	Stirred tank	70–100		0	Nitric acid (4–7%)	n.a.	
Laboratory	Preoxidized kraft lignin	0·4-liter batch autoclave	100, 190	150	0·1	NH_4OH (8%)	11·8	
Laboratory	Kraft lignin impregnated with cations	Fluidized bed	210			Ammonia/air (1·75/1)	18	

LS: lignosulfonate; SSL: spent sulfite liquor; n.a.: not available.

2.3.2 Characteristics of oxidative ammonolysis

Oxidative ammonolysis is characterised by the reaction of an oxidative degradative oxygen species with lignin as a polymer of a particular molecular mass distribution in an alkaline medium in the presence of the nucleophile ammonia under increased temperature and pressure as illustrated in the scheme in figure 12 below.

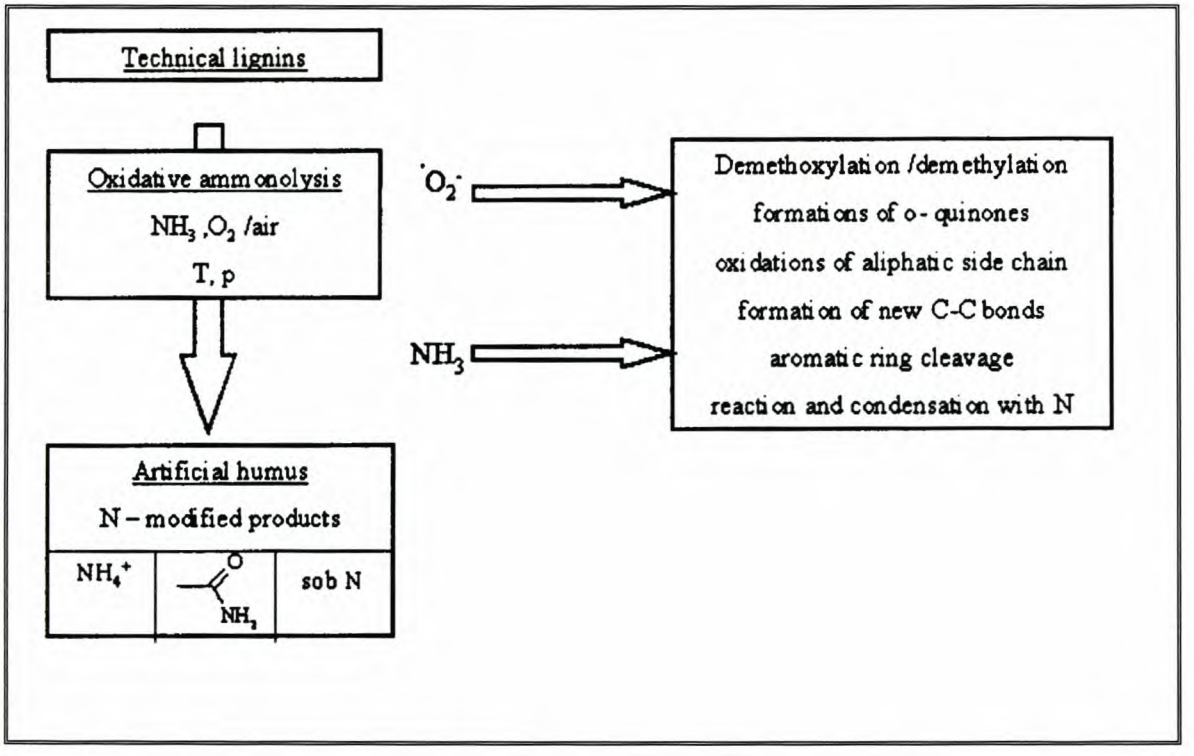


Figure 12: Reaction scheme for the artificial humification of technical lignins **[3].**

This process can take place under high-pressure conditions according to the high-pressure principle (HPP), as well as under normal pressure according to the normal pressure principle (NPP), as observed by Schiene et al [48]. These workers, in their early investigations used sulphite-spent liquors for the production of *N*-lignins with fertilising ability. From these studies, they observed that oxidative ammonolysis is a two-step process. While the first step involves alkaline initiated fragmentation of the lignin macromolecule which is accompanied by demethoxylation, cleavage of ether bonds and of aromatic rings, the second step involves the introduction of nitrogen in several bonding types e.g. as ammonium salts, as amides or amines up to the formation of heterocycles. It could also be shown that in the course of oxidative ammonolysis, a number of chemical reactions are involved in the conversion of macromolecular lignin sulphonates, which comprise the degradation of OCH_3 groups and desulphonation. Subsequent to the alkaline nitrobenzene oxidation of *N*-lignins produced

under both high and normal pressure conditions, vanillin, syringaldehyde and the respective carboxylic acids can be verified as oxidation products.

From these studies, it could also be concluded that in both the high and low-pressure processes, oxidative ammonolysis is accompanied by condensation reactions towards low molecular mass substances. This is more pronounced in the harsher high-pressure compared to the milder low-pressure process. It was also observed that oxidative ammonolysis according to the NPP leads to lower nitrogen contents N_t (14,1 – 15,2%) and Sob-N proportions of (35 – 56,3%) as compared to the HPP (N_t = 25%, Sob-N = 70,8%). When pot tests were conducted applying lignins as fertilisers, detrimental effects on yield occurred due to optimum exceeding concentrations, which were obviously caused by physiological active substances. Hence, it followed that during oxidative ammonolysis of spent sulphite liquors under the HPP as well as in certain reaction conditions with the NPP, substances were formed which are physiological active in plants, and which as shown on pot tests inhibit the growth of seedlings. These effects were the result of low-molecular mass heterocyclic reaction products formed in the oxidative ammonolysis of monosaccharides. A conclusion that the properties of *N*-lignins are affected by both reaction parameters and composition of parent waste liquors was therefore made. In subsequent studies, Schiene et al [49] used organosolv lignins, which resulted from low polluting process of pulping, which in addition to being sulphur free, were easily separable from the process spent liquors as well as having a high degree of purity and uniformity. From these studies, it was found that N-lignins obtained from organosolv lignins, when compared to those obtained from sulphite-spent liquors were different in relative N distribution i.e. organosolv lignins had a lower amount of NH_4^+-N but higher contents of NH_2-N and sob-N. Organocell lignins had higher reactivity at lower temperatures compared to spent sulphite liquors. Process optimisation trials also showed that the reaction parameters, temperature, oxygen supply/pressure and lignin concentration had a dominant effect on N-modification. Added to this, material properties of the parent lignins, such as variations in the modes of precipitation from spent liquors also had an effect. This was further confirmed by Meier et al [47] in their comparative studies with technical lignins derived from kraft, organosolv, soda and ASAM pulping as well as a fermented lignosulphate from a sodium sulphite process with the aim of producing a slow nitrogenous fertiliser. The effects of time and temperature, time and oxygen pressure on the amount of fixed nitrogen and C/N ratios were studied. 13 – 14% nitrogen (based on lignin) could be fixed in the lignin macromolecules with high nitrogen contents being obtained with kraft and organosolv lignins followed by the lignosulphonate. Studies have also been conducted by Rozmarin et al **[50]** using lignocellulosics as raw material. Latest studies by Capanema et al **[51]** on the kinetics of

the reaction under isothermal conditions have shown that the reaction pathways did not change during the course of oxidative ammonolysis. Oxygen pressure was shown to have a linear and direct influence on lignin solubilisation, implying that the rate of lignin degradation directly depends on oxygen pressure. The nitrogen incorporation was also linearly correlated with oxygen uptake, CO_2 formation, oxygen incorporation into the lignin and loss of methoxyl group content. This suggests that the reaction proceeds via the same pathway under different kinetic conditions **[52]**. When linear correlation between nitrogen incorporation into the lignin and molecular oxygen uptake, CO_2 formation, O-dimethylation and total carbon loss, were analysed at different temperatures, it was found that temperature affects the reaction rate and not the reaction pathway **[53]**.

2.3.3 Reaction pathways of oxidative ammonolysis

The aim of oxidative ammonolysis of technical lignins is to synthesise organomineral *N*-fertilisers with high nitrogen contents with C/N ratios in the range between 10 and 12 as well as a good distribution of the various binding forms of nitrogen. In this regard it is important to understand the chemical reactions taking place during the oxidative ammonolysis of lignins, in particular in comparison with the formation of humic substances in nature. In this regard, an array of reaction pathways has to be assumed. Structural investigations on N-modified lignins by Potthast et al **[54]** using a combination of analytical methods including NMR spectroscopy in particular ^{15}N-NMR, IR spectroscopy, and GC/MS analysis have shown that substructures such as aromatic nitriles (see figure 13) and urea were constituents of *N*-modified lignins and *N*-modified lignin model compounds. Figure 14 shows a possible pathway for the formation of aromatic nitriles by oxidative ammonolysis under high pressure. A number of low molecular weight fragments with ring-bonded nitrogen) were identified as well as some amides. *N*-heterocyclic compounds were not found. However nitrogen compounds bonded to aromatic rings, were found (see figure 15).

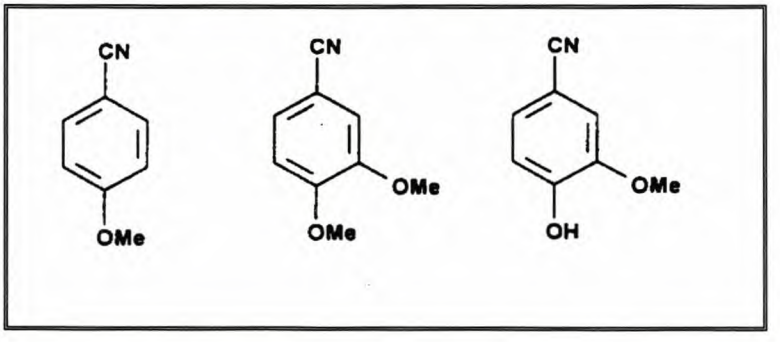


Figure 13: Nitriles produced by N-modification of lignin model compounds **[54]**

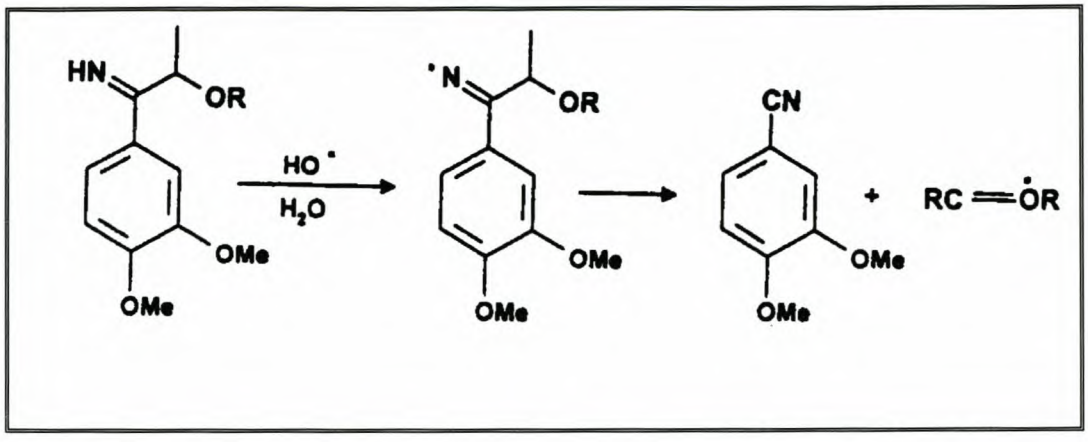


Figure 14: Possible pathway for the formation of aromatic nitriles by oxidative ammonolysis **[54]**

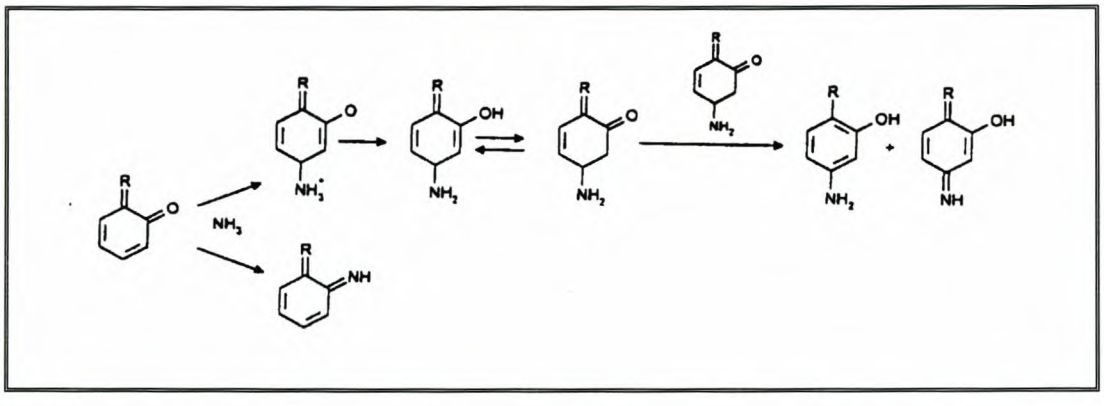


Figure 15: Ring bonded nitrogen compounds and the possible pathways for their formation **[54]**

2.3.4 Characteristics of *N*-lignins

N-lignins have favourable characteristics such as their ability to function as organic fertilisers. They are active over several growing periods (see figure 16) analogous to the natural humus containing various types of bonds i.e.

- ammonium nitrogen which is the easily and short term sulobilizable form of nitrogen and hence immediately available to plants;
- amide nitrogen, the mid term solubilizable nitrogen and finally the
- strongly organically bonded nitrogen (Sob-N) which is solubilized over a long term.

Tests conducted by various workers including those conducted by the Institute for Post Mining Landscapes, Finsterwalde, Germany as well as phytochamber experiments at The Institute of Plant and Wood Chemistry of the Technical University if Dresden in Germany have shown that *N*-lignins have the ability to decrease *N*-leaching to the ground water (see

figure 17) in addition to imparting decontaminating effects in contaminated soils (see figure 18) **[3]**.

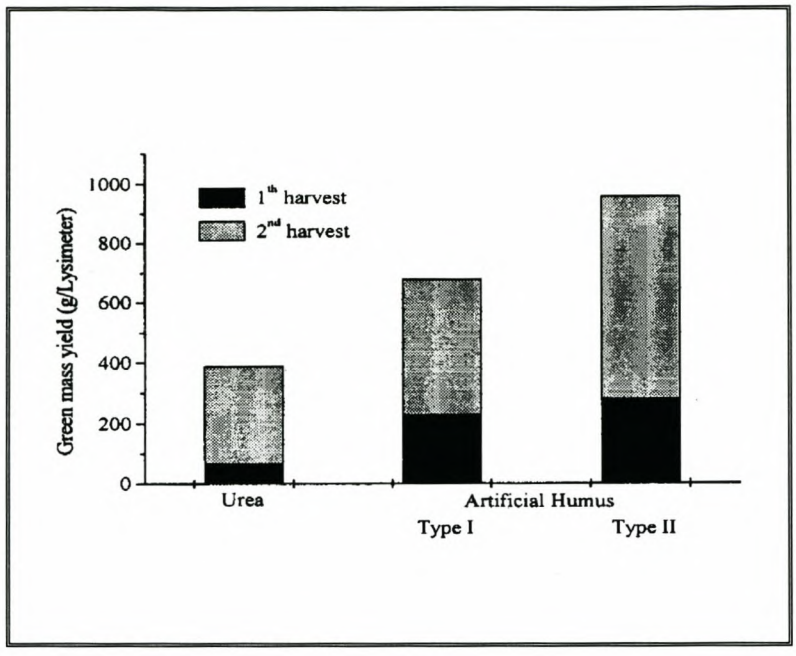


Figure 16: Long term effect of artificial humus **[3]**.

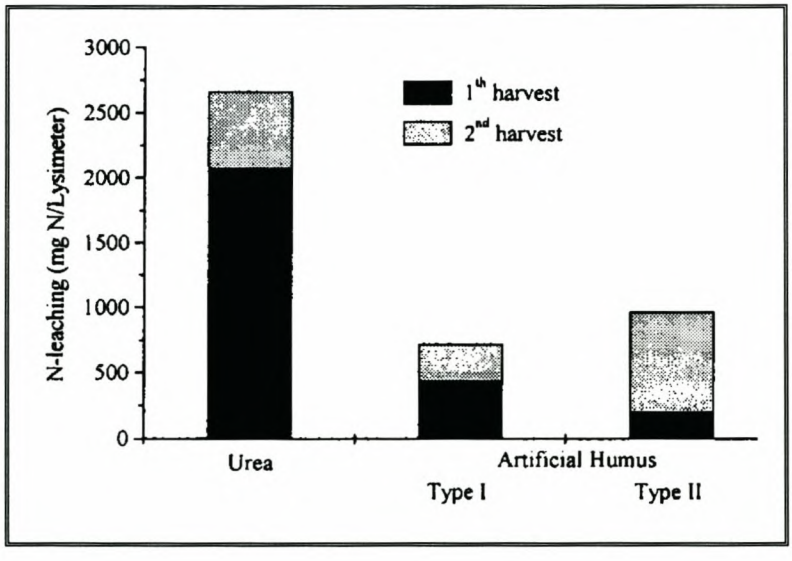


Figure 17: Decrease of N-leaching by artificial humus **[3]**

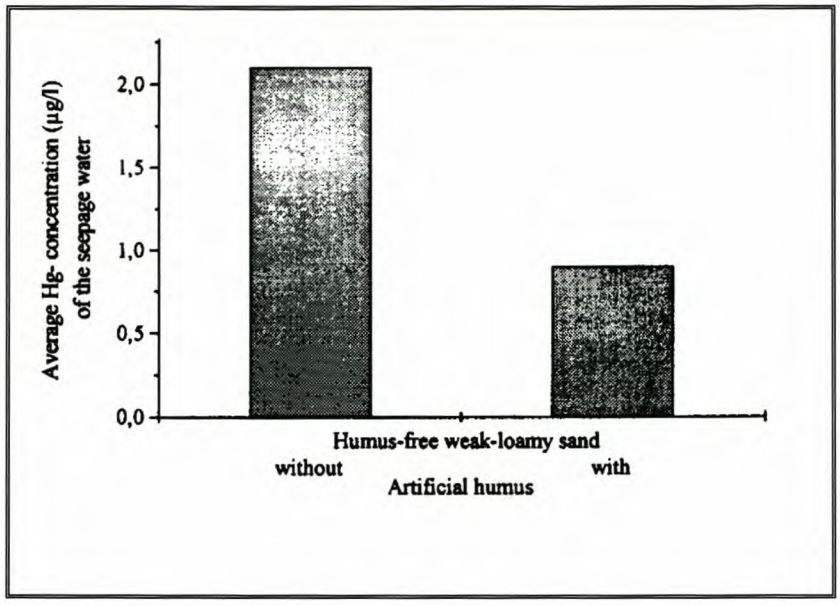


Figure 18: Immobilisation of toxic heavy metal Hg by artificial humus **[3]**

Gonzalez et al **[55]**, in their tests of ammoxidized kraft lignins as a slow nitrogen release fertilizer on *Sorghum vulgare* in pot experiments found that in comparison to ammonium sulphate as a conventional inorganic fertiliser, modified lignins were a satisfactory source of nitrogen when applied in high dosage, producing similar grain and biomass yields as the reference. The soil fertilized with *N*-functionalised lignins showed essentially lower amounts of NO_3^- in the percolation water than soil fertilised with ammonium sulphate. In comparison with natural humus, there are similarities in the basic reactions and this permits a discussion of analogies despite distinct differences. Oxidative ammonolysis in vitro applies harsher conditions that drastically shorten the reaction time in contrast to the humification process that takes place in nature. The biogenic organic matter is converted in nature. In vitro however, the parent material is technical lignin, which has already been altered by the pulping process and contains low quantities of admixed substances. Analogies are found in the macromolecular building principle of the aliphatic linked aromatic units, in the N-functional groups in the form of amide, nitrile and amine functionality including heterocyclic structures. There are similarities between the C and N contents (thus C/N ratios) as well as the distribution of N-binding forms (% and N-functional groups, the latter detectable by cationic exchange (CEC)). The artificial humus (N-lignins) contain fewer acid groups, thus their cationic exchange is lower than that of natural humic substances. Table 3 below illustrates the structural properties of artificial and natural humus.

Table 3: Comparison between the structural properties of artificial and natural humus [3]

Humus	C%	N_{tot} %	NH_4^+-N %	Amide-N %	Sob N %	OCH_3 %	CEC
Artificial	49 – 59	3 – 5	10 – 41	14 – 21	44 – 73	10 – 12	140 – 180
Natural	41 – 62	1 – 5	10 – 25	21 – 45	Ca. 50	0.9 – 1.8	180 – 500

2.3.5 Summary

Soil organic matter is a key component of soils systems. Its function is to provide plants with nutrients via processes of decomposition of plant and animal material and humus formation. It is also important for maintaining proper nutrient levels. Soils devoid of organic matter are less attractive economically than soil systems with adequate organic matter. It is in this regard that processes for the manufacture of novel type humic substances such as oxidative ammonolysis have been developed. The *N* functionalised lignins and lignites, which are produced through this process, have a humus like structure, a feature on which their application is based. Their differentiated release of nitrogen, analogous to humus makes them active over several growing seasons. In addition to this they have decontaminating effects as been discussed above. They also have an advantage over inorganic fertilisers as they application does not result in ground water pollution.

CHAPTER 3

EXPERIMENTAL METHODS

3.1 MATERIALS

3.1.1 Raw materials from South Africa

3.1.1.1 Technical lignins

- A lignin from Smithchem Development Department, Umbogintwini, South Africa with a trade name "Sucrolin". The lignin is obtained from sugar cane bagasse by way of auto-hydrolysis delignification.
- A lignocellulosic residue from Smithchem Development Department, Umbogintwini, South Africa.
- A calcium lignosulphonate obtained from Lignotech Sappi Joint Venture, Umkomaas, South Africa.

3.1.1.2 Lignites

- A Neogene, probably a Miocene lignite from Kraaifontein, 25 km east of Cape Town, South Africa. The lignite is generally used for horticultural purposes.
- A Neogene, probably a Miocene lignite from Cramix Brick Field, Brackenfell, Cape Town, South Africa.

3.1.2 Raw materials from Germany

The following materials were used for the production of novel type humic substances. They were also used as the basis for comparison with the South African materials:

3.1.2.1 Technical lignin

A commercial Kraft lignin with a trade name INDULIN AT™ from Westvaco, USA.

3.1.2.2 Lignites

A Miocene lignite from the Lusatian Lignite Mining district located 80km south of Berlin, Germany.

3.1.3 Chemicals

All chemicals were obtained from commercial chemical suppliers in South Africa and Germany. The following chemicals were used:

- Chemically pure, laboratory grade ammonium hydroxide solution with a minimum assay of 25%.
- Technical-grade oxygen gas from Afrox.
- Analytical grade hydrogen peroxide solution with a minimum assay of 30%.

3.2 ANALYTICAL METHODS

3.2.1 CHARACTERISATION OF TECHNICAL LININS AND LIGNITES

3.2.1.1 Elemental analysis

The raw materials and N-modified products were analysed using the following method:

Instrument:	Vario (Elemental Analysensysteme Hanau)
Weighing scale:	Mikrowaage MC 500 (Satorius)
Calibration:	Sufanylic acid; control of blind value and daily correction factor every morning; the blind value must be zero and daily factor 0,9<factor<1.1
Carrier gas:	Helium N 4.6
Oxidation gas:	Oxygen N 4.5

General principle

The sample was packed airtight in tin foil and burnt in oxygen flow at 1150°C. Water was adsorbed on Siccapent (Merck-Nr.543). The excess oxygen and formed nitrogen oxides were given to react with copper to form copper oxide and nitrogen gas. The working temperature of the copper was 850°C. The halogens formed eventually were removed by silver wool. N_2, CO_2 and SO_2 were subsequently detected in a thermal conductivity cell. Their separation was carried out by use of adsorption columns. Each of the gases was adsorbed when the measuring cell had reached the baseline value again. Therefore, this equipment provided a consequent single detection of each compound.

3.2.1.2 Structural characterisation of technical lignins and lignites

3.2.1.2.1 General principle

Most of the methods used for structural analysis of organic polymers involve volatilisation of the materials prior to injection in the Gas Chromatograph. One of the most convenient and frequently used method is the Curie-Point Pyrolysis GC/MS. This method employs standardized chemical or thermo-chemical degradation processes. The degradation processes result in the fragmentation of the polymers. The structure of the polymers can be deduced from the fragments so formed. Pyrolysis-GC/MS therefore belongs to deductive methods for structural analysis. The Curie-Point Pyrolysis-GC/MS instrument is shown in Figure 19 below.

Figure 19: A Curie point Pyrolysis Gas Chromatograph coupled to a Mass Spectrophometer

The Curie-Point pyrolyser unit: Combined with a gas chromatograph, the pyrolyser serves as a sampling system. Its function is to convert the sample into a complex mixture of gaseous products which exhibit the characteristics of the original polymer. Pyrolysis is achieved in the absence of oxygen and at a defined working temperature. Curie-Point Pyrolysis takes place under reproducible conditions, which are required for quantitative evaluation. A sample container made of a special alloy is heated within a few milliseconds to the Curie point temperature. This is the temperature where the alloy loses its magnetic properties. The inductive heating results from a high frequency voltage. The choice of the pyrolysis temperature has a great influence on the intensity of the pyrolytic degradation of the material. It is, therefore, mostly set to maximize the proportion of gaseous low molecular mass

compounds and also to maintain the degradation as mild as possible so as to obtain maximum structural information. In the analysis, the components are separated by means of gas chromatography. A good separation of the components of a polymeric compound is obtained by optimising the pyrolysis period. At first, secondary reactions, which would result in substantial complications regarding the interpretation of the chromatograms, are avoided by using short times of pyrolysis. On the other hand it is necessary to apply longer periods of pyrolysis to yield representative mixtures of analyte in cases where the samples have low specific heat conductivity.

The capillary gas chromatography unit: The gaseous mixture of analyte was injected into the inlet of a gas chromatograph after pyrolysis had taken place. The transfer of the pyrolysis products is done with the help of a carrier gas through heated transfer pipes. The choice of the chromatographic conditions (geometry of the capillary column, stationary phase, velocity of the transfer gas, temperature program) was chosen so as to achieve the separation of a maximum number of compounds of the complex mixture at the baselines.

The mass sensitive detector unit: The separated components of the complex gaseous mixture are ionised (70eV) by electron impact ionisation. The resulting molecular fragment ions are detected by means of a mass sensitive detector using the mass charge ratios (secondary electron multiplier). The separated components are identified using a spectral library. The information obtained from the spectral library is used to deduce the original structures of the polymers **[56]**.

3.2.1.2.2 Experimental parameters

Pyrolysis Gas Chromatograph coupled to a Mass Spectrophotometer

Pyrolyser:	(CPP-40 Curie Point Pyrolyser system from Fischer/GSG).
Alloy:	Fecralloy (Goodfellow)
Temperature:	600°C
Carrier gas:	Helium
Sample amount:	200-1000 µg
Purge time:	5s
Pyrolysis time:	10s

Gas Chromatograph:	(GC 6890, Agilent Technologies)
Carrier gas:	Helium
Inlet temperature:	250°C split less
Carrier gas velocity:	0.9ml/min
Temperature:	50°C (5 min) to 280°C (5°C/ min), 280°C (2 min)
Auxiliary temperature:	150°C
Mass Spectrophotometer:	(MSD 5973, Agilent Technologies)
Ionisation method:	EI (70 eV), 200°C, 1.5×10^{-5} Torr
Detector:	Photomultiplier
Identification:	(HP CHEM-STATION)
Spectral Library:	NIST 2000

3.2.1.3 Analysis of the different nitrogen binding forms

3.2.1.3.1 General principle

The three different nitrogen binding forms i.e. ammonium nitrogen, amide nitrogen and the strongly organic bonded nitrogen were determined from all starting materials and products from each experiment were determined according to Kjeldah method for total nitrogen analysis. The basic method is a two-step process. The first step is the addition of a base to the sample to form ammonia. The second step is the transportation of ammonia by steam into a flask, which contains a standard HCl and an indicator. When the ammonia formation is finished, the acid is back titrated with a standard NaOH solution.

3.2.1.3.2 Experimental

Ammonium Nitrogen (NH^+_4-N) determination

150-180mg of sample material was placed in a round bottom flask. 2g of MgO and 50cm^3 of water were added to the flask. 10 cm^3 of standard 1 M HCl solution was transferred to a 250 cm^3 Erlenmeyer flask and 3 drops of methyl red indicator were added. The bottom flask was connected to a steam generator. This reaction was carried out for 15 minutes. The acid was

back titrated with a standard 1M NaOH solution. The NH^{+}_{4}-N was then determined as follows:

$$[NH_4^+ - N] = \frac{(acid\ [ml] - base\ [ml]) \times 1.40067}{(mass\ [g])} \times 100\ \% \quad (1)$$

1.40067 is the correction factor for the M/10 concentration of the base used.
Mass [g] is the mass of the sample in grams

Amide Nitrogen (NH_2-N) determination

In this, experiment a more concentrated NaOH (42M) solution and a longer reaction time (45 minutes) were used. These conditions were severe enough to release not only the loosely bound NH^{+}_{4}-N, but also the stronger bound NH_2-N. The total NH_2-N was then determined as follows:

$$[NH_2 - N] = \frac{[acid\ (ml) - base\ (ml)] \times 1.40067 \times 100\ \%}{(mass\ [g])} - [NH_4^+ - N] \quad (2)$$

150-180mg of sample material was placed in a round bottom flask. 15 cm^3 of a 45 M NaOH solution followed by 45 cm^3 of water were added. 10 cm^3 of standard HCL (1 M in this case) was placed in a 250 cm^3 Erlenmeyer flask with 3 drops of methyl red indicator. The round bottom flask, which contained the sample, was connected to steam generator. Then the NH_3 was transferred by steam into the 250ml Erlenmeyer flask containing the standard HCl solution and the indicator. The reaction was carried out for 45 min. The acid was then back titrated with a standard NaOH (also1 M) base.

The strongly organic bonded nitrogen (Sob-N)

The strongly organic bonded nitrogen (Sob-N) was determined by the following equation:

$$[Sob - N] = N_{tot} - [(NH_4^+ - N) + (NH_2 - N)] \quad (3)$$

N_{tot} is the total nitrogen determined by the elemental analyser.

3.2.2 OXIDATIVE AMMONOLYSIS

Oxidative ammonolysis was carried out on the laboratory and pilot plant scales, with the ultimate objective to carry out the process on an industrial scale. Oxidative ammonolysis basically involved reacting the substrate material with ammonia (the source of nitrogen) in the presence of oxygen, which acted as an oxidising agent under increased heat and pressure.

3.2.2.1 Oxidative ammonolysis on a lab-scale

Two types of laboratory scale systems were used for this project i.e. a bench scale autoclave system and a Büchi model BEP 280 stirred laboratory-autoclave system. The difference between these two systems was in the design and capacity. The operating principle and conditions under which they operated were exactly the same.

3.2.2.1.1 Reaction parameters

Sample concentration:	10% solids (mass%/volume%)
Ammonium hydroxide concentration:	5% (V%/V%)
Temperature:	80°C
Reaction time:	5hours
Oxygen pressure:	7.5 bar

3.2.2.1.2 Equipment

3.2.2.1.2.1 The Bench Scale Autoclave System

The bench-scale autoclave system was constructed of stainless steel. It was composed of two units i.e. the reaction vessel and a cap with an oxygen inlet, which was connected to the oxygen source by means of rubber tubes. The cap was also equipped with a pressure gauge to record the pressure inside the vessel during the reaction stage. The reaction vessel had a capacity of holding 50ml of sample. Heat was supplied by placing the reaction vessel in an oil-bath, which had been pre-heated to the desired reaction temperature. Mixing of the reaction components was achieved by means of a magnetic stirrer. Stirring was always kept at 1000rpm. Oxygen was supplied from the top through a cap at a specified pressure. The reaction was performed by placing the material in a solution to a final volume of 40ml (to

avoid overflowing of the reaction components) inside the reaction vessel. The components were mixed manually until all the material was in solution. After the reaction, the vessel was allowed to cool. The pressure was relieved. The reaction components were transferred into a Petri dish and the aqueous ammonia was allowed to evaporate in a fume cupboard. The dry samples were prepared for further processing.

3.2.2.1.2.2 The Büchi model BEP 280 stirred laboratory-autoclave system

The autoclave system was fitted with a double wall CrNiMo-steel cylindrical reaction vessel with a hemispherical bottom. Its maximum capacity was 5 litres, maximum operating pressure 40 bars and the maximum operating temperature 250 °C. The reaction vessel was coupled by means of a set of braided steel hoses to a Brabender T 300A circulating heating unit with a built-in electronic proportional temperature control with a maximum operating temperature of 260°C. Grade B Shell Thermia oil was used for heating. The reaction temperature was measured by means of a PT 100 sensor fitted to the reactor lid and coupled to an external measuring device of the heating unit. The reaction was carried out by pre-mixing and dissolving the material to a final volume of 4 litres inside the reaction vessel. Pre-mixing was achieved by using a power drill, fitted with a stirrer. The drill and the vessel each were held in position by swivel arms, both attached to a stand (see figure 20). A special PVC holder mounted on a table supported the reactor vessel and the whole assembly was able to slide on rails. Pre-mixing could also be done in a separate container and the reaction components could be transferred into the reaction vessel thereafter. After completion of the pre-mixing, the table and the whole assembly was slid underneath the reactor lid, elevated with a lab jack and the vessel tightly bolted to the lid with nuts, where after the reaction commenced (see figure 21). A spiral stirrer (see figure 22) was used to agitate the reaction components. Stirring was kept at 1000rpm. The whole reaction system was placed in an extraction hood.

Figure 20: The reactor in the pre-mixing stage **(57)**

Figure 21: The reactor in the reaction mixing stag **(57)**

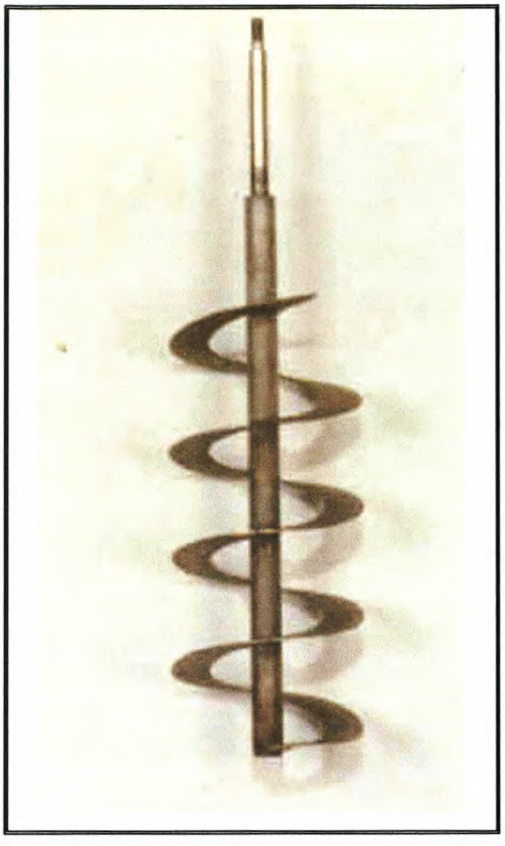


Figure 22: A spiral stirrer which is used to agitate the reaction components.

After completion of the reaction, the product was collected at the bottom of the reactor system into a container. It was poured in a large tray to allow the aqueous ammonia to evaporate. The samples were prepared for further testing.

3.2.2.2 Oxidative ammonolysis at pilot-plant scale

A small-scale plant, located in Freienhufen about 100km from Tharandt was commissioned in January 2000 and in April 2000, after initial testing, it was used to manufacture the *N*-modified lignin/lignite required for field trials. The pilot plant facility consists of a mixing tank for the reactants, reactor, a tank for the reaction products, an ammonium washer, and an ammonia recovering system. The oxidative ammonolysis reaction was computer controlled. Data from process variables could be downloaded on a computer for further evaluation. This process, contrary to the laboratory-scale *N*-modification, took place under normal pressure conditions. Oxygen was fed continuously to the reactor.

3.2.3 PRE-ACTIVATION OF THE RAW MATERIALS

Investigations were carried out to investigate whether pre-oxidation of the starting material before *N*-modification by oxidative ammonolysis could have any influence on the amount of nitrogen incorporated on the material. For this purposes two substances with a lignin oxidising (and degrading ability) were used i.e.:

(i) The white rot fungus C*hrysosporium phanaerochateae,* and
(ii) hydrogen peroxide under different reaction conditions.

3.2.3.1 Pre-activation with *Chrysosporium phanaerochateae*

Chrysosporium phanaerochateae is a white rot fungus, which specifically degrades lignin and leaves cellulose and hemicelluloses intact in wood. It does this by secreting an enzyme called lignin peroxidases. This enzyme, besides being a powerful oxidizing agent, seems to control the C-C and C-O bond splitting in primary radical cations of substrates. It also appears to contribute to the oxidative-hydrolytic dealkylation of radical cations. Theoretically, oxidation can play a vital role in the incorporation of nitrogen during the process of oxidative ammonolysis. Therefore, the total amount of nitrogen that would be incorporated after this experiment was expected to be more compared to that of the material, which had not been pre-oxidised. Of the starting materials, only the lignocellulosic residue, the lignites from Kraaifontein, Germany, and the Indulin AT™ were used for this experiment.

Experimental

5g of each of the starting materials was placed and moistened in a Petri dish, which had perforations that facilitated gaseous exchange between the contents of the Petri dish and the outside atmosphere. 10ml of a *Chrysosporium phanaerochateae* fungal suspension was inoculated into each sample material. Triplicates were made for each material. Each sample was moistened with 10ml of tap water and was left at room temperature for a week. Samples were moistened once with 5ml of water to facilitate fungal growth. After a week, a number of samples with a good fungal growth were selected. From each material, a small amount of more than 7g was dried in preparation for oxidative ammonolysis and further investigations.

3.2.3.2 Pre-activation with hydrogen peroxide

H_2O_2 is used to increase pulp brightness during pulp bleaching processes. It does so by breaking down the lignin closely associated with cellulose. The action of the hydrogen peroxide under alkaline conditions as a bleaching agent is explained through the reactions of the perhydroxyl anion, HO_2^- which is formed according to the equilibrium:

$$H_2O_2 + OH^- \rightarrow HO_2^- + H_2O$$

This anion is believed to be the principal active species involved in the elimination of chromophores in lignin structures, particularly carbonyl structures that are prone to react with the perhydroxyl anion. The aim of this experiment was to eliminate some of these carbonyl structures in the lignin substances with the perhydroxyl anion, with the aim of exposing more bonding sites for the nitrogen. Theoretically, oxidation plays a vital role in the incorporation of nitrogen during the process of oxidative ammonolysis. Therefore the total amount of nitrogen incorporated after this experiment was expected to be more, compared to the material which had not been pre-oxidised.

Experiment 1:

Reaction parameters

Material used:	Autohydrolysis lignin (Sucrolin)
Hydrogen peroxide concentration:	1%, 3% and 5% in water (V%/V%)
Solid content:	10% solids concentration in hydrogen peroxide solution (mass%/volume%)
Temperature:	60°C (constant)
Reaction time:	4hrs

Experimental

400g of the material was dissolved in an alkaline hydrogen peroxide solution and pre-mixed inside the Büchi model BEP 280 stirred laboratory-autoclave system. After pre-mixing, the reactor was closed and the reaction was started using the reaction parameters described below. The stirring was kept at 1000rpm. After the reaction, the material was transferred into a large tray and allowed to dry in a fume hood. When dry, it was *N*-modified according to the procedure described in section 3.2.2.1. The pre-oxidised material and the *N*-modified products

were prepared for structural characterisation, elemental analysis and the determination of the various *N*-binding forms as outlined in section 3.2.1.3.

Experiment 2

Reaction parameters

Material used:	Lignocellulosic residue, Indulin AT™, lignite ex Kraaifontein and Germany
Hydrogen peroxide concentration:	10% in water (V%/V%)
Solid content:	2% solids concentration a hydrogen peroxide solution (mass%/V%)
Temperature:	Room temperature
Reaction time:	4hrs

Experimental

5 g of each of the starting materials were placed in a 250ml water suspension (a 500ml Erlenmeyer flask was used). 100ml of 10% (V%/V%) hydrogen peroxide was added to the suspension. The solution was stirred for 4 hours. It was then transferred to a Petri dish and allowed to dry. 4g of each material were ammonolysed at lab scale as outlined in section 3.3.1. The pre-oxidised material and the *N*-modified products were characterised for elemental composition structure and the different *N*-binding forms as outlined in section 3.2

CHAPTER 4

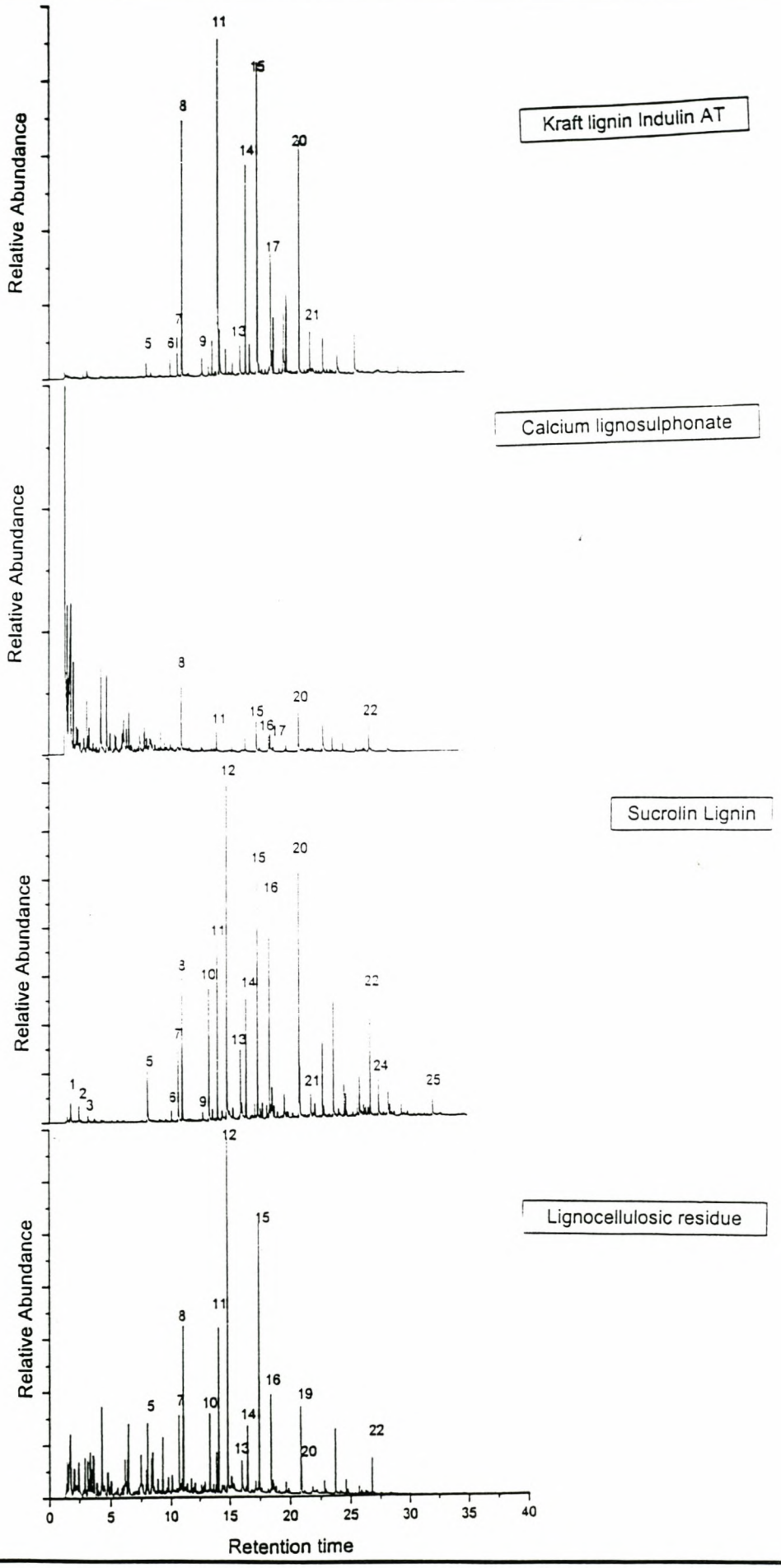
RESULTS AND DISCUSSION

4.1 STRUCTURAL CHARACTERISATION OF TECHNICAL LIGNINS AND LIGNITES BY CURIE-POINT PYROLYSIS GC-MS

Analytical pyrolysis of the technical lignins and lignites yielded the typical monomeric products 1–25 summarized in tables 4 and 5. These products, which are mainly characteristic of the raw materials used in the manufacture of the novel type humic substances, were selected and used as the basis for comparison between the South African materials and the German materials. Figures 23 and 24 show the pyrograms of the various technical lignins and lignites. The numbering of peaks refers to the compounds listed in tables 4 and 5. The lignosulphonate as shown in figure 23 showed a different structural composition when compared to the other three materials. Of the selected monomeric products, only a few were found in this material. Their abundance was not that high (see also table 4). The high sulphur content (4%) was also characteristic of this material, which in the pyrogram is represented by the highest peak and hence high relative abundance. The differences between the other three materials can be described in terms of relative abundance and the types of monomeric products that were found in each material. This is shown in table 4. Figure 24 shows the pyrograms obtained after the pyrolysis of the various lignites. The difference in structural compositions of the four materials is visually presented from these pyrograms. The main series of compounds detected from the South African lignites were aliphatic moieties. The aromatic pyrolysis products were few to almost non-existent (see also table 5).

Figure 23: (overleaf). Total ion chromatograms of the thermal degradation products obtained after pyrolysis of technical lignins. The numbering of the peaks refers to the compounds listed in tables 4 and 5.

Figure 24: (overleaf). Total ion chromatograms of the thermal degradation products obtained after pyrolysis of the lignites. The numbering of the peaks refers to the compounds listed in tables 4 and 5. (* Contaminant)



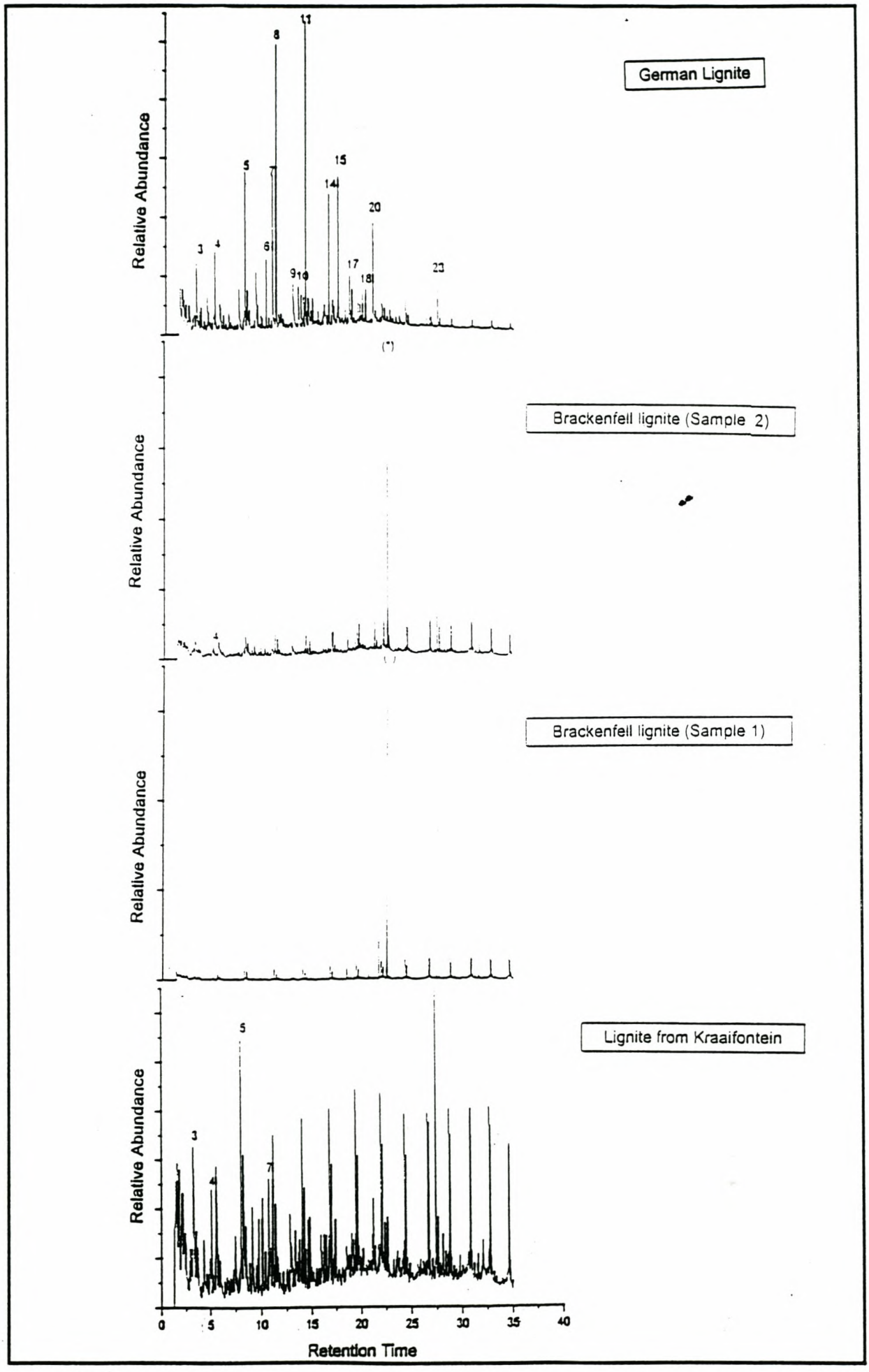


Table 4: Pyrolysis products derived from technical lignins

Peak no	Retention time	Compound	MW	Assigned Structure*	Sucrolin lignin	Kraft lignin	Lig.cellcResidue	Lignosulphonate
1	1.8	2-Methylfuran	82	F-CH_3 (2)	+	-	-	-
2	2.49	2,5-Dimethylfuran	96	F-CH_3 (2,5)	+	-	-	-
3	3.14	Toluene	92	B-CH_3	+	-	-	-
4	5.1	p-Xylene	106	B-CH_3(1,4)	-	-	-	-
5	8.02	Phenol	94	P	+	+	+	-
6	10.17	Phenol,-2-methyl-	108	P-CH_3 (2)	+	+	-	-
7	10.71	Phenol, 4-methyl-	108	P-CH_3 (4)	+	+	+	-
8	11.1	Phenol, 2-methoxy-	124	P-OCH_3 (2)	++	+++	++	+
9	12.83	Phenol,2,4-dimethyl-	122	P-CH_3 (2,4)	+	+	-	-
10	13.35	Phenol, 4-ethyl-	122	P-CH_2-CH_2- (4)	++	-	++	-
11	14.1	Phenol, 2-methoxy-4-methyl-	138	G-CH_3	++	+++	++	+
12	14.86	2,.3-Dihydrobezofuran	120	B-O-$(CH_2)_2$ (2,3)	+++		+++	-
13	16	1.2-Benzenediol,3-methoxy	140	B-(OH)(1,2)-OCH_3(3)	+	+	+	-
14	16.48	Phenol, 4-ethyl-2-methoxy-	152	G-CH_2-CH_3 (4)	++	++	+	-
15	17.43	2-Methoxy-4-vinylphenol	150	G-CH=CH_2 (4)	+++	+++	+++	+
16	18.42	Phenol, 2,6-dimethoxy	154	P- $(OCH_3)_2$ (2,6)	+++	-	++	+
17	18.59	Eugenol	164	G-CH_2-CH=CH_2 (4)	-	++	-	+
18	20.21	Naphthalene,2,7-dimethyl-	156	N-(CH_3)-(2,7)	-	-	-	-
19	20.867	3 Hydroxy-4-methoxybenzoic acid	168	B-(OH)(COOH)(3,4)	-	-	++	-
20	20.93	Phenol, 2-methoxy-4- (1-propenyl)-	164	G-CH=CH-CH_3 (4)	+++	++	+	+
21	21.84	Ethanone,1-(4-hydroxy-3 methoxyphenyl)-	166	G-CO-CH_3 (4)	+	+	-	+
22	26.81	3-Hydroxy-4-methoxybenzoic acid	168	B-(OH)(COOH)(3,4)	++	-	+	-
23	27.38	1-Heptene,2-isohexyl-6-methyl	196		-	-	-	+
24	27.53	Ethanone,1-(4-hydroxy-3,5-dimethoxyphenol)-	196	P-C=O$(CH)_3$) (OH) $(OCH_3)_2$(1,4,3,5)	+	-	-	-
25	32.11	n-Hexadecaonic acid	82		+	-	-	-

[a] F (Furan), B (Benzene) P (phenol), G (Guaiacyl) and N (Napthalene). Not detected (-); low, medium, high abundances (+, ++, +++). * the number in brackets refers to carbon number in the benzene ring.

Table 5: Pyrolysis products derived from lignites

Peak no	Retention time	Compound	MW	Assigned Structure	German lignite	Kraaifontein	Brackenfell 01	Brackenfell 02
1	1.8	2-Methylfuran	82	F-CH_3 (2)	-	-	-	-
2	2.49	2,5-Dimethylfuran	96	F-CH_3 (2,5)	-	-	-	-
3	3.14	Toluene	92	B-CH_3	+	+	-	-
4	5.1	p-Xylene	106	B-CH_3(1,4)	+	+	-	+
5	8.02	Phenol	94	P	++	+	-	-
6	10.17	Phenol, -2-methyl-	108	P-CH_3 (2)	+	-	-	-
7	10.71	Phenol, 4-methyl-	108	P-CH_3 (4)	++	+	-	-
8	11.1	Phenol, 2-methoxy-	124	P-OCH_3 (2)	+++	-	-	-
9	12.83	Phenol,2,4-dimethyl-	122	P-CH_3 (2,4)	+	-	-	-
10	13.35	Phenol, 4-ethyl-	122	P-CH_2-CH_2- (4)	+	-	-	-
11	14.1	Phenol, 2-methoxy-4-methyl-	138	G-CH_3	+++	-	-	-
12	14.86	2,3-Dihydrobezofuran	120	B-O-$(CH_2)_2$ (2,3)	-	-	-	-
13	16	1.2-Benzenediol,3-methoxy	140	B-(OH)(1,2)-OCH_3(3)	-	-	-	-
14	16.48	Phenol, 4-ethyl-2-methoxy-	152	G-CH_2-CH_3 (4)	++	-	-	-
15	17.43	2-Methoxy-4-vinylphenol	150	G-CH=CH_2 (4)	++	-	-	-
16	18.42	Phenol, 2,6-dimethoxy	154	P- $(OCH_3)_2$ (2,6)	-	-	-	-
17	18.59	Eugenol	164	G-CH_2-CH=CH_2 (4)	+	-	-	-
18	20.21	Naphthalene,2,7-dimethyl-	156	N-(CH_3)-(2,7)	+	-	-	-
19	20.867	3 Hydroxy-4-methoxybenzoic acid	168	B-(OH)(COOH)(3,4)	-	-	-	-
20	20.93	Phenol, 2-methoxy-4- (1-propenyl)-	164	G-CH=CH-CH_3 (4)	++	-	-	-
21	21.84	Ethanone,1-(4-hydroxy-3methoxyphenyl)-	166	G-CO-CH_3 (4)	-	-	-	-
22	26.81	3-Hydroxy-4-methoxybenzoic acid	168	B-(OH)(COOH)(3,4)	-	-	-	-
23	27.38	1-Heptene,2-isohexyl-6-methyl	196		+	-	-	-
24	27.53	Ethanone,1-(4-hydroxy-3,5-dimethoxyphenol)-	196	P-C=O$(CH)_3$) (OH) $(OCH_3)_2$(1,4,3,5)	-	-	-	-
25	32.11	n-Hexadecaonic acid	82		-	-	-	-

[a]F (Furan), B (Benzene) P (phenol), G (Guaiacyl) and N (Naphthalene). Not detected (-); low, medium, high abundances (+, ++, +++). Brackenfell 01 and Brackenfell 02 refer to samples 1and 2 from Brackenfell.
* the number in brackets refers to carbon number in the benzene ring.

4.2 ELEMENTAL COMPOSITION OF TECHNICAL LIGNINS AND LIGNITES AND THEIR N-MODIFIED PRODUCTS

4.2.1 Technical Lignins

4.2.1.1 Raw materials

The elemental compositions of the technical lignins used for oxidative ammonolysis are presented in table 6. These materials were analysed in two laboratories with two different instruments. One instrument lacked the capability of measuring sulphur and the other lacked the capability of measuring hydrogen. In the tables, the elements, which could not been analysed, are indicated with a dash. The most important differences are in the carbon contents. Calcium lignosulphonate and the lignocellulosic residue had lower carbon contents (up to 18%) when compared to the Sucrolin and the Kraft lignins. The high sulphur content of the lignosulphonate is quite expected as well.

Table 6: Elemental analysis of the technical lignins (based on oven dry mass)

Lignin	Dry matter (mass%)	Carbon* (mass%)	Nitrogen (mass%)	Sulphur (mass%)	C/N ratio
Sucrolin	95.63	65.79	0.79	-	83.24
Kraft	97.70	62.90	1.17	2.11	53.90
Lignosulphonate	94.94	43.30	0.14	4.70	309.3
Lignocellulosic Residue	95.70	48.85±0.09	0.33±0.02	0.26±0.28	149.65±6.16

* the number with ± is the standard deviation

(-) Not analysed for this element.

4.2.1.2 Products form oxidative ammonolysis on a lab scale

The results for the oxidative ammonolysis of technical lignins are presented in table 7. As the table demonstrates, the technical lignins differed considerably in their ability to bind nitrogen. The highest nitrogen incorporation was achieved with Sucrolin after oxidative ammonolylsis. A low C/N ratio (<6) also resulted with this material (see table 7). In comparison, very low nitrogen contents were obtained with the other 3 lignin substances. This was more so with the lignocellulosic residue, where total nitrogen content of less than 2% was obtained.

The low nitrogen contents were associated with high C/N ratios, once again, more so for the lignocellulosic residue, where a C/N ratio of 34 was achieved. This therefore means that relative to the carbon content, this material has low nitrogen content and hence the biological degradation of the humic matter derived from it will be very slow. A good C/N ratio would be found in the range between 10 and 12. Kraft lignin showed more nitrogen binding ability, compared to calcium lignosulphonate.

Table 7: Elemental analysis of the lab scale *N*-modified technical lignins (based on oven dry mass) using the Büchi model and the bench scale-autoclave systems

Lignin	Dry matter (mass%)	Carbon (mass%)	Nitrogen (mass%)	Sulphur (mass%)	C/N ratio
Sucrolin (Büchi model, Stellenbosch)	84.76±4.12	53.51±2.44	10.05±0.77	-	5.34±0.463
Kraft (Autoclave, Germany)	89.50	61.60	3.93	1.90	15.70
Lignosulphonate (Autoclave, Germany)	90.71±1.42	42.04±0.40	3.25±0.54	4.51±0.07	13.19±2.47
Lignocellulosic Residue (Autoclave, Germany)	94.4±0.29	50.10±0.25	1.44±0.07	0.12±0.03	34.25±1.86

* the number with ± is the standard deviation

(-) Not analysed for this element.

Previous studies carried out by Reimann **[58]** on Sucrolin have shown that this material is more reactive than Kraft lignin, which perhaps explains its better response towards oxidative ammonolysis. Studies by Meier et al **[47]** on oxidative ammonolysis have shown that Kraft lignins were more reactive than lignosulphonates. Perhaps this confirms the slightly lower reactivity of the lignosulphonate when compared to the Kraft lignin. The low reactivity of the lignocellulosic residue can possibly be explained by the fact that only a fraction of this material contained lignin. Perhaps the conditions for oxidative ammonolysis were too moderate to dissolve the cellulosic material

4.2.1.3 Products form oxidative ammonolysis on a pilot plant-scale

4.2.1.3.1 Kraft lignin and the lignocellulosic residue

The oxidative ammonolysis results of Kraft lignin and the lignocellulosic residue are presented in table 8. According to these results, no significant differences could be observed when the pilot plant process was compared to the lab scale process.

Table 8: Elemental analysis of the pilot-scale *N*-modified technical lignins (based on oven dry mass)

Lignin	Dry matter (mass%)	Carbon Content (mass%)	Nitrogen Content (mass%)	Sulphur Content (mass%)	C/N ratio
Kraft	96.7	60.00	3.07	1.76	19.50
Lignocellulosic Residue	97.6±0.29	51.60±0.25	2.35±0.07	0.12±0.03	22.00±1.70

4.2.1.4 Elemental analysis of products subjected to pre-activation prior to oxidative ammonolysis on a lab scale

4.2.1.4.1 Fungal pre-activation

The raw materials used for this experiment were Kraft lignin and lignocellulosic residue. The results of oxidative ammonolysis are presented in table 9 below. A slight increase in nitrogen content from 1.44% (unactivated material) to 1.58%, as can be seen form the table, was achieved with the lignocellulosic residue. In contrast to the lignocellulosic residue, no significant difference was observed with the Kraft lignin. Since the effect of pre-oxidation by the fungal species so was minimal, no significant changes were observed in the C/N rations of both materials.

Table 9: Elemental composition of the fungal inoculated technical lignins (based on air dry matter)

Lignin	Dry matter (mass%)	Carbon Content (mass%)	Nitrogen Content (mass%)	Sulphur Content (mass%)	C/N ratio
Kraft lignin	90.50	60.20	3.74	1.81	16.10
Lignocellulosic residue	97.10	47.7	1.58	0.05	30.30

When the two materials were inoculated with the fungal suspension, more fungal growth was observed with the lignocellulosic residue as can be visually observed on figure 26. This was because the lignocellulosic material absorbed more water when moistened, when compared to the Kraft lignin. This therefore, made it easy for the fungi to grow on this material.

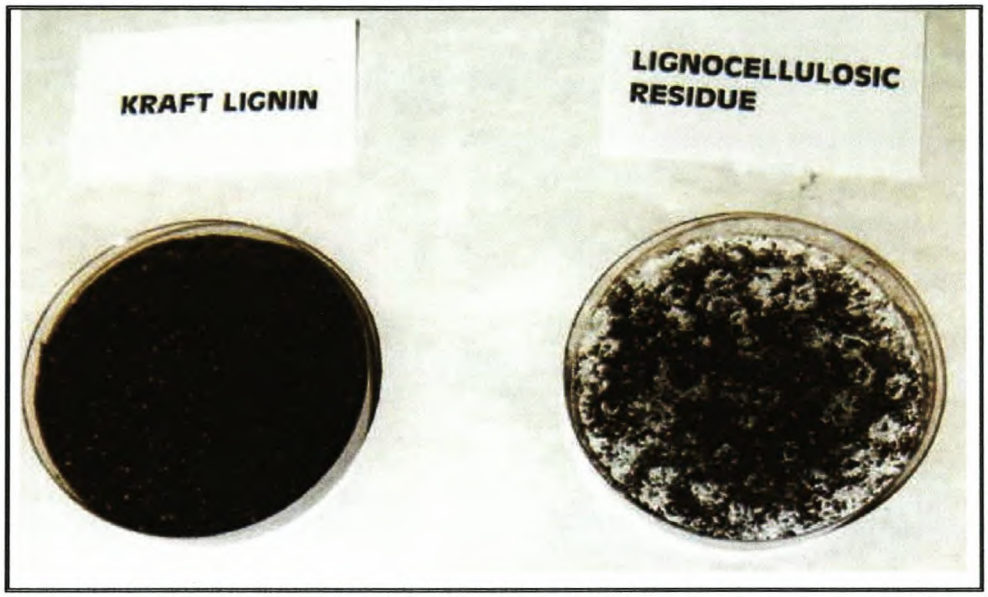


Figure 25: Fungal pre-activated Kraft lignin and lignocellulosic residue samples.

The sugar origin of the lignocellulosic material also meant that there was more easily digestible for the fungi as compared to the Kraft lignin.

4.2.1.4.1 Pre-activation with hydrogen peroxide

The results for the H_2O_2 pre-activated and lab-scale *N*-modified Sucrolin lignin are presented in table 10. These results suggest improvement in the total incorporated nitrogen after pre-oxidation. It can also be seen that nitrogen incorporation improved with the increase in the concentration of H_2O_2.

Table 10: Elemental compositions of the H_2O_2 pre-activated *N*-modified Sucrolin lignin (based on air dry matter)

H_2O_2 (v%/v%)	Dry matter (mass%)	Carbon Content (mass%)	Nitrogen Content (mass%)	C/N ratio
0	84.76±4.12	53.51±2.44	10.05±0.77	5.34±0.46
1	85.36± 1.05	57.07± 0.02	11.80± 0.28	4.84 ± 0.12
3	86.52± 0.02	58.00± 0.84	10.11± 0.27	5.74 ± 0.24
5	84.67 ± 5.04	56.76 ± 3.54	12.70± 0.34	4.45 ± 0.16

This is further suggested by the results obtained with lignocellulosic residue (see table 11). Pre-activation of this material with 10% hydrogen peroxide yielded a nitrogen content that is more than two times the amount achieved with un-activated material.

Table 11: Elemental compositions of the Lignocellulosic residue after pre-activation with H_2O_2 and *N*-modification at a laboratory scale (based on dry organic matter)

H_2O_2 (v%/v%)	Dry matter (mass%)	Carbon Content (mass%)	Nitrogen Content (mass%)	Sulphur Content (mass%)	C/N ratio
0	94.4 ± 0.29	50.10±0.25	**1.44 ± 0.07**	0.12 ± 0.03	**34.25±1.86**
10	90.30	49.20	**3.72**	0.09	**13.20**

The increase in nitrogen content after pre-activation was also associated with C/N ratios closer to the acceptable range (10-12).

4.2.2 Lignites

4.2.2.1 Raw materials

The elemental compositions of the lignites used for oxidative ammonolysis are presented in table 12. The South African lignite samples were found to have less than half the total carbon content of the German lignite samples. The slightly higher sulphur content from the Kraaifontein lignite was another notable difference when compared to the German lignite.

Table 12: Elemental composition of lignite raw materials (based on air dry matter)

Lignite	Dry matter (mass%)	Carbon Content (mass%)	Nitrogen Content (mass%)	Sulphur Content (mass%)	C/N ratio
Germany	89.60	62.90	0.71	0.83	88.00
Kraaifontein	85.90	28.60	0.30	2.29	94.60
Brackenfell 01	84.98	21.24	0.37	-	61.43
Brackenfell 02	81.00	22.20	0.30	0.42	75.00

(-) Not analysed for this element.

The low carbon content can be attributed to the fact that the South African lignites have a high mineral ash content, which can be up to 45.80% as found by Cole and Roberts **[59]**. The carbon content of humic matter in the natural environment is in the range between 41 – 62%. In artificial humus it is between 49 – 65%. Therefore the low carbon content of the South African lignites suggested that the *N*-modified products obtained from them would have low nutritional value.

4.2.2.2 Products from oxidative ammonolysis on a lab scale

The oxidative ammonolysis of the lignite substances yielded the results presented in table 13. There was no significant difference observed in the C/N ratios of the various materials. However, low nitrogen contents were achieved with the South African lignites. The lignite from Kraaifontein gave slightly better results in this respect when compared to the Brackenfell material.

Table 13: Elemental composition of lab-scale *N*-modified lignites (based on dry organic matter)

Lignite	Dry matter (mass%)	Carbon Content (mass%)	Nitrogen Content (mass%)	Sulphur Content (mass%)	C/N ratio
Germany (Autoclave Germany)	87.05	62.8	5.05	0.85	12.40
Kraaifontein (Autoclave Germany)	92.50	34.30	3.81	1.97	9.00
Brackenfell 01 (Autoclave Stellenbosch)	92.81± 0.73	25.23± 1.52	2.78± 0.20	-	9.07± 0.28
Brackenfell 02 (Autoclave Stellenbosch)	95.05± 0.21	23.4± 1.27	2.05± 0.06	-	11.5± 0.28

4.2.2.3 *Products from oxidative ammonolysis on a pilot plant-scale*

4.2.2.3.1 Experiments with German and Kraaifontein lignites

The pilot plant scale results for oxidative ammonolysis of these lignites are presented in table 14. Compared to the lab scale process, the pilot plant scale evaluation yielded slightly higher nitrogen contents with the German lignite, with an increase from 5.05% to 6.16% in total nitrogen content. However, it was the opposite for the South African lignite material, with the total nitrogen content decreasing from 3.81% to 3.46%.

Table 14: Elemental composition of pilot plant *N*-modified lignites (based on air dry matter)

Lignite	Dry matter (mass%)	Carbon Content (mass%)	Nitrogen Content (mass%)	Sulphur Content (mass%)	C/N ratio
Germany	87.80	60.00	6.16	0.85	12.40
Kraaifontein	98.10	46.70	3.46	2.14	13.50

No great differences in the C/N rations were recorded, but as observed for the lab scale modification, the South African lignite was characterized by low nitrogen and carbon contents. The carbon content of this material did increase somewhat after both modification processes from 28.6% (raw material) to 34.30% at lab scale and to 46.7% at a pilot plant scale.

4.2.2.4 Pre-activation of raw materials prior to oxidative ammonolysis

4.2.4.1 Fungal pre-activation

Fungal growth could not be observed on the lignite substances under the conditions employed. As can be seen in figure 27 (section 4.2.1.4.1), the inoculums remained where they were initially placed and did not spread in any of the materials. Perhaps a possible explanation for this is the difficulty in moistening the materials due to their hydrophobic nature.

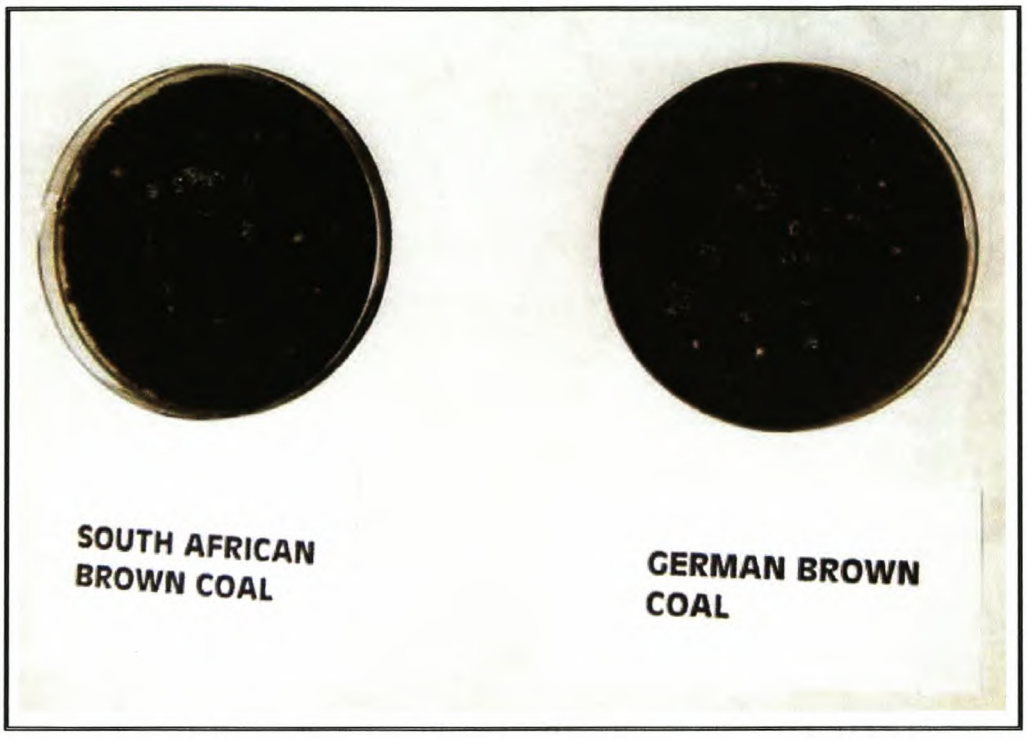


Figure 27: Fungal pre-activated South African lignite from Kraaifontein, and Germany samples.

4.2.4.2 Pre-activation with hydrogen peroxide

The elemental compositions of the hydrogen peroxide activated lignites are presented in table 15. The increase in total nitrogen contents for both materials after activation with H_2O_2 is clearly visible from the table. The decrease in the C/N ratio as well is another noteworthy observation. The sulphur contents on the other hand seemed to increase. This is more so for the South African lignite.

Table 15: Elemental composition of hydrogen peroxide activated lignites (based on air dry matter)

Lignite	H_2O_2 Content (v%/v%)	Moisture Content (mass%)	Carbon Content (mass%)	Nitrogen Content (mass%)	Sulphur Content (mass%)	C/N ratio
Germany	**0**	87.05	62.8	5.05	0.85	12.40
	10	85.20	50.10	9.59	0.88	5.20
Kraaifontein	**0**	92.50	34.30	3.81	1.97	9.00
	10	93.70	26.70	5.46	3.07	4.90

4.3 ANALYSIS OF THE DIFFERENT BINDING FORMS OF NITROGEN

4.3.1 Technical lignins

4.3.1.1 Non-activated materials

Table 16 presents the analytical results of the different binding forms of nitrogen i.e. **ammonium nitrogen, amide nitrogen** and the **strongly organically bonded nitrogen**. The results for the starting materials are also given in order to indicate the state of the materials prior to *N*-modification. The three binding forms are given as a percentage of the total nitrogen (N_t).

Table 16: The three main classes of *N*-binding forms of nitrogen of technical lignins

Lignin	**N_t (mass%)**	**NH_4^+-N [a] (mass%)**	**Amide-N [a] (mass%)**	**Sob-N [a] (mass%)**
Sucrolin lignin				
Raw material	0.79	7.59	6.33	86.08
N-modified material	**10.05±0.77**	**35.58±1.55**	**15.10±3.38**	**49.32±4.90**
Kraft lignin				
Raw material	1.17	76.10	6.80	17.10
N-modified material	**3.07**	**62.70**	**14.00**	**23.30**
Lignocellolosic residue				
Raw material	0.315	30.40	10.10	59.50
N-modified material	**2.35±0.18**	**17.90±0.50**	**16.50±0.28**	**65.60±0.21**
Calcium lignosulphonate				
Raw material	0.14	35.70	0	64.29
N-modified material	**3.25±0.54**	**48.30±1.20**	**10.32±2.35**	**41.30±3.89**

[a] % of N_t

The most notable observations from the results concern the Kraft lignin and the lignocellulosic residue. A total of **62.7 %** of the ammonium nitrogen was determined in the Kraft lignin. In comparison, the lignocellulosic nitrogen had only **17.9 %** ammonium nitrogen. Correspondingly, the differences in Sob-N of the two materials were very high i.e. **23.3 %** for the Kraft lignin and almost **66%** for the lignocellulosic residue. The Sucrolin

lignin and the calcium lignosuphonate showed a more appropriate distribution of the three *N*-binding forms as outlinen in page 35. However, the calcium lignosulphonate had **12.72 %** more ammonium nitrogen compared to the Sucrolin lignin and correspondingly **8 %** less Sob-N.

4.3.1.2 Hydrogen peroxide pre-activated materials

4.3.1.2.1 Sucrolin lignin

Table 17 shows the nitrogen binding forms of a hydrogen peroxide activated Sucrolin lignin. No remarkable difference was observed in the nitrogen binding forms of the inactivated (see control sample) and activated samples.

Table 17: The three main classes of *N*-binding forms of hydrogen peroxide pre-activated and modified Sucrolin lignin

H_2O_2 (v%/v%)	N_t (mass%)	NH_4^+-N [a] (mass%)	Amide-N [a] (mass%)	Sob-N [a] (mass%)
0	10.05	35.58	15.10	49.32
1%	11.80	34.87 ±0.06	11.70 ±0.81	53.44 ±0.02
3%	10.11 ±0.27	38.45 ±2.05	13.06 ±0.49	48.50 ±2.13
5%	12.73	34.76	11.30	53.95

[a] % of N_t

4.3.2 Lignites

The nitrogen binding forms of all four lignite materials are presented in table 18. As can be seen, the German lignite had a more equal distribution of all the *N*-binding forms with a total Sob-N of 56.2 %. In comparison, the South African lignites (both Kraaifontein and Brackenfell) are characterized by high amounts of the immediately available nitrogen (ammonium nitrogen). There is little variation in the total amide nitrogen for the South African lignites. However, the values are lower than those obtained for the German lignite.

Table 18: The three main classes of *N*-binding forms of the *N*-modified lignites

Lignite	**N_t (wt%)**	**NH_4^+-N [a] (wt%)**	**Amide-N [a] (wt%)**	**Sob-N [a] (wt%)**
Germany				
Raw material	0.63	28.60	1.60	69.80
N-modified material	**5.05**	**35.50**	**11.30**	**56.20**
Kraaifontein				
Raw material	0.30	40	6.70	53.30
N-modified material	**3.46**	**60.9**	**6.10**	**33.00**
Brackenfell 01				
Raw material	0.39	0	50	50.00
N-modified material	**2.78 ±0.20**	**62.14 ±8.81**	**7.33 ±2.95**	**30.63 ±9.60**
Brackenfell 02				
Raw material	0.30	50.00	50.00	0
N-modified material	**2.05 ±1.18**	**57.46 ±0.35**	**7.59 ± 2.64**	**34.96 ±1.70**

[a] % of N_t

CHAPTER 5

CONCLUSION

5.1 TECHNICAL LIGNINS

In this study, it was shown that slow release nitrogenous fertilizers, similar to the novel type humic substances produced by the Institute of Plant and Wood Chemistry of the Technical University of Dresden in Germany, could be produced using South African produced technical lignins as raw materials. This was more evident as was shown by the results obtained with Sucrolin lignin, whereby a total nitrogen content of 10% (about 5% higher than that of the materials produced in Germany) was observed. The nitrogen binding forms distribution of this material was also within the range obtained with other artificial humic materials such as the novel type humic substances i.e. NH_4^+-N; 10 - 41%; Amide-N, 14 - 21% and Sob-N, 44 - 73%. In contrast to these results, significantly lower nitrogen contents were obtained with the lignocellulosic residue (1.44%). The C/N ratio of 34 obtained with this material is far too high for this material to be used as a fertilizer. In comparison, the oxidative ammonolysis of the Kraft lignin and the calcium lignosulphonate resulted in slightly higher nitrogen contents (3.93 % and 3.72% respectively).

No significant differences in nitrogen content were observed between oxidative ammonolysis products from the lab scale and pilot plant processes.

This study also showed that pre-activation of the raw material had beneficial effects on nitrogen content that is incorporated after the oxidative ammonolysis reaction. This was especially so with hydrogen peroxide pre-activation, where beneficial effects could be observed at concentrations as low as 1%. This, however, seemed to be more beneficial to materials which already had high nitrogen contents. The activation with fungal cultures requires further investigation, as there was no significant change in nitrogen contents of the materials after oxidative ammonolysis. The lignocellulosic residue showed better water-absorbing capacity than the Kraft lignin and hence better fungal growth was observed. However, a longer incubation period is required in order to establish the effect of the fungal inoculation on this material.

5.2 LIGNITES

The South African lignites exhibited a different structural composition compared to the German lignite. The main constituents of the South African raw materials were

aliphatic in nature. These lignites also had high ash contents. The carbon content of these materials was also very low i.e. 23 – 30% (almost half and less than the German lignites, which had 62.8%). When these materials were subjected to oxidative ammonolysis, the lignite from Kraaifontein showed a better N content (3.81% compared to 5.05% of the German material) compared to the material from Brackenfell (2 - 2.78%). The carbon content of this material was also slightly higher (30%) and the nitrogen content was comparable to that of the Kraft lignin. The use of hydrogen peroxide to activate these materials prior to oxidative ammonolysis resulted in improved nitrogen contents. However, due to the general low quality of this material as well as that of the material from Brackenfell (ash content and structural composition), a search for more suitable lignite deposits needs to be conducted.

5.3 SUMMARY AND RECOMMENDATIONS

5.3.1 The oxidative ammonolysis of the Sucrolin lignin yielded better results when compared to the calcium lignosulphonate and the lignocellulosic residue. This material therefore needs to be produced in sufficient quantities (perhaps on a pilot plant scale) to conduct pyhtochamber tests and perhaps field trials.

5.3.2 The calcium lignosulphonate yielded lower nitrogen contents when compared to the Sucrolin lignin. The application rates of this material can therefore be expected to be high. Investigations need to be carried out to detremine if pre-oxidation with oxidising agents such as hydrogen peroxide can be used to improve the nitrogen content. The economical feasibility of producing this material needs to be investigated.

5.2.3 A search for high quality lignite deposits needs to be conducted.

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